DIFFERENTIAL TOLERANCE OF TOMATO (*Lycopersicum esculentum* Mill.) AND PEPPER (*Capsicum annuum* L.) TO RIMSULFURON AND HALOSULFURON HERBICIDES

By

RICHARD STEELE BUKER III

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Abstract of Dissertation Presented to the Graduate School
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DIFFERENTIAL TOLERANCE OF TOMATO (*Lycopersicum esculentum* Mill.) AND PEPPER (*Capsicum annuum* L.) TO RIMSULFURON AND HALOSULFURON

By

Richard Steele Buker III

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Rimsulfuron and halosulfuron are sulfonylurea herbicides that will control many common weeds in tomato and pepper production. Tomato demonstrates excellent tolerance to these herbicides, but at similar rates pepper has exhibited severe injury. The objectives for this research focused on the characterization of tomato tolerance to rimsulfuron and halosulfuron, by comparison with the sensitive species pepper.

Nine tomato cultivars ('BHN 444,' 'Captiva,' 'Carolina Gold,' 'Celebrity,' 'Cherry grande,' 'Equinox,' 'FL 47,' 'FL 91,' and 'Mountain Spring') were tested for tolerance to rimsulfuron and halosulfuron up to 0.08 kg ai/ha. All cultivars exhibited excellent tolerance to rimsulfuron and halosulfuron applied at labeled rates. 'BHN 444,' 'FL 47,' and 'Carolina Gold' were grown out to yield, without reductions in any cultivars. The application of oxamyl insecticide plus rimsulfuron or halosulfuron did not reduce the shoot weight of any of the nine cultivars. Furthermore, the yields of 'BHN 444,' 'FL 47,' and 'Carolina Gold' were not reduced from halosulfuron and oxamyl combinations.

The target site sensitivity, uptake, translocation, and metabolism of halosulfuron and rimsulfuron was tested in tomato and pepper were tested. The inhibition constants of halosulfuron and rimsulfuron with tomato and pepper acetolactate synthase (ALS) were similar. Tomato and pepper exhibited similar root uptake of halosulfuron (1.7 versus 01.4 nmoles/hr) and rimsulfuron (0.7 and 1.1 nmoles/hr). Foliar uptake of halosulfuron was 0.3 and 0.1 nmoles/hr in tomato and pepper, respectively. Rimsulfuron foliar uptake was 0.56 nmoles/hr in both species. Translocation was less than 8% in both species 72 hours after treatment (HAT).

The majority (63 to 93%) of rimsulfuron absorbed by tomato was metabolized within 24 HAT. This is contrasted by very low metabolism in pepper (87 to 97% of parent compound remained 24 HAT). Abiotic breakdown was less than 20% in all experiments. Based on these data, transformation influenced by metabolic activity appears to be the primary mechanism by which tomato tolerates rimsulfuron.

CHAPTER 1
INTRODUCTION

Florida has historically been the second largest producer and supplier of fresh market tomato and pepper in the United states (National Agricultural Statistics Service, 2001). Tomato and pepper are grown throughout the state. Production of these vegetables is dependent on the use of methyl bromide. Use of this soil fumigant has been widely adopted by Florida vegetable growers, because of its spectrum of pest control and flexibility in application. Methyl bromide controls nematodes, pathogens, and weeds, but more importantly it can be applied shortly before planting (~ 1 week) with no negative residual effect on the crop. Furthermore, at the labeled rate methyl bromide will usually provide adequate soil borne pest control for two growing seasons. Currently 98% of Florida tomato and pepper growers use polyethylene mulch and methyl bromide to control soil borne pests (Florida Agricultural Statistics Service, 2001).

Methyl bromide was determined in the Montreal Protocol to be a class I ozone depleting material and is scheduled to be banned for agricultural use by the year 2005 (United Nations Environment Program, 1992). Research efforts continue to search for chemical alternatives to replace methyl bromide. To date it appears that Telone C-17® (a mixture of chloropicrin and 1,3-dichloropropene) is the most viable option (Gilreath et al., 1998). It will control most nematodes and pathogens that are troublesome in Florida tomato and pepper production, but does not provide control of many problem weed species (Gilreath et al., 1998). If growers continue to use polyethylene mulch, then nutsedge species are expected to be the primary weed problem. Preliminary trials using polyethylene mulch without methyl bromide have demonstrated the nutsedge problem with yield losses up to 50% being reported (Gilreath et al., 1998). Herbicides will be necessary without the use of methyl bromide.

Trials have been conducted in Florida with the objective of identifying herbicides that will control weeds, especially nutsedge species, and not cause unacceptable yield loss to tomato or peppers. Two herbicides identified for weed control in tomato are rimsulfuron and halosulfuron. Both herbicides provide excellent nutsedge activity when applied to the soil or foliage. Tomato exhibited tolerance to these herbicides applied either preemergence (PRE) or postemergence (POST) (Stall, 1999; Morales-Payan et al., 1999). Pepper was not tolerant to these herbicides when applied POST, while PRE tolerance of these herbicides is variable (Stall, 1999; Ackley et al., 1998). Ackley et al. (1998) reported poor tolerance to rimsulfuron when applied PRE, while Stall (1999) reported less injury when applied PRE compared to POST.

Differential tolerance of tomato and pepper to rimsulfuron and halosulfuron present a challenge for future pepper production and a curious academic question. Tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*) are both in the Solanaceae family, and plants within a taxonomic family tend to respond similarly to herbicides (Radosevich et al., 1997). To understand why pepper is intolerant of these herbicides one must start with known mechanisms of plant tolerance to herbicides. Mechanisms of plant herbicide tolerance have been thoroughly reviewed (Lua and O'Keefe, 1996; Radosevich et al., 1997; Devine et al., 1993). There are several factors that influence herbicide tolerance in plants. Herbicide metabolism and lack of sensitivity to the herbicide through altered target enzymes are the most common determinants (Lua and O'Keefe, 1996; Radosevich et al., 1997; Devine et al., 1993). Isozymes (target and metabolic enzymes), herbicide rate applied, stage of plant development, and environmental conditions can also influence plant tolerance. (Lua and O'Keefe, 1996; Radosevich et al., 1997; Devine et al., 1993).

In a preliminary study by Stall (1999), it seems unlikely that the differential tolerance between tomato and pepper to pre emergence applications of sulfonyl ureas is due to the differences in rooting structure. Growth stage did not affect the tolerance in either species, since tomato was tolerant to PRE and POST applications of rimsulfuron and halosulfuron, while pepper was intolerant. Rate influenced the response of tomato to rimsulfuron. At rates greater than 280 g ai/ha tomato loses its tolerance and yield losses occur (Morales-Payan, 1999; Stall, 1999). This is not the case for pepper, at all rates examined there was unacceptable yield losses with rates that control weeds (Ackley et al., 1998; Stall, 1999). A previous study conducted by Stall (1999) compared tomato and pepper tolerance under the same environmental conditions, and concluded environmental influences on the differential tolerance between these species can be negated.

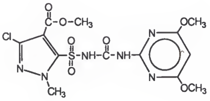
These results suggest that the differential tolerance between these species to these herbicides is based on differences in physiological activity. There is no published literature providing any indication of the mechanism of tolerance of tomato to either rimsulfuron or halosulfuron. Furthermore, there is no literature published describing why peppers are susceptible to halosulfuron and rimsulfuron. The objectives of this research project were to determine the mechanism of tolerance of tomato to halosulfuron and rimsulfuron, and thereby to infer the physiological basis of peppers susceptibility.

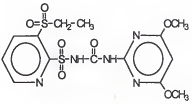
CHAPTER 2
REVIEW OF LITERATURE

The herbicidal properties of sulfonylureas (SU) were discovered in 1975 by George Levitt (Brown and Cotterman, 1994). Since their discovery there have been over 20 SU herbicides labeled for use (Anonymous, 2001; Brown and Cotterman, 1994). The large number of SU developed can be attributed to two factors. First, these herbicides were deemed valuable because of their low use rates, low impact on flora and fauna, and flexibility in application (Brown and Cotterman, 1994). The large variation of SU herbicides is related to their unique structure. Their basic structure is composed of a pyrimidine ring attached to a pyridine or pyrazole ring by a SU bridge (Gee and Hay, 1994). Many different analogs can be attached to the heterocyclic rings, thereby affecting the biological activity of the compound.

SU inhibit the enzyme acetolactate synthase [ALS (EC 4.1.3.18)]. This enzyme is not found in animals, which lowers any potentially negative impact from this chemistry. The rates of SU used in crop production range from 0.002 to 0.150 kg/ha of active ingredient. The highest rate used (for non-crop areas) is 0.680 kg/ha of active ingredient, which is a typical use rate for many commonly used herbicides in crop production. The many possible analogs for the basic structure of a SU alters the spectrum of biological activity, but in most cases it does not appear to influence the uptake and translocation properties. All commercially available SU herbicides are reportedly systemic herbicides. Most SU are absorbed from root and shoot tissue and translocated throughout plants. There are some SU that exhibit limited foliar uptake and translocation. Foliar adsorption of triflusulfuron is slow (<10% in 20 hours), and translocation out of the leaf is also limited (Anonymous, 1994).

Rimsulfuron and Halosulfuron





Rimsulfuron (N-[[[4,6dimethoxy-2-pyrimidynl]amino]carbonyl]-3-[ethylsulfonyl]-2-pyridinesulfonamide) and halosulfuron ([methyl 5-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonylaminosulfonyl]-3-chloro-1-methyl-1-H-pyrazole-4-carboxylate) are SU herbicides characterized by a pyridine and a pyrazole ring, respectively, attached by a sulfur bridge to a pyrimidine ring. Both rimsulfuron or halosulfuron exhibit herbicidal activity when applied to either the foliage or soil of sensitive plants.

Rimsulfuron is currently labeled for use in maize (0.005 to 0.015 kg/ha), potatoes (0.005 to 0.015 kg/ha), soybeans, and processing tomatoes. Rimsulfuron is only available as a pre-mix for use in maize, under the trade name Basis® or Basis Gold®. In potatoes and processing tomatoes, it is available as 25% ai dry flowable, under the trade names of Matrix® and Shadeout®, respectively. Rimsulfuron provides control of an extensive spectrum of broadleaf weeds (Palm et al., 1989). Rimsulfuron controlled common cocklebur (*Xanthium strumarium* L.), cutleaf groundcherry (*Physalis angulata* L.), common ragweed (*Ambrosia artemisiifolia* L.), flixweed (*Descurainia sophia* L.), hairy nightshade (*Solanum sarrachoides* Sendtner), ivyleaf morningglory (*Ipomoea hederacea* L.), kochia (*kochia scoparia* L.), redroot pigweed (*Amaranthus retroflexus* L.), smooth pigweed (*Amaranthus hybridus* L.), tumble pigweed (*Amaranthus albus* L.), and shepherd's purse (*Capsella bursa-pastoris* L.), ≥85%, with the rate of 0.035 kg/ha POST (Ackley et al., 1996; Robinson et al., 1996; Blackshaw et al., 1995; Eberlein et al., 1994; Mekki and Leroux, 1994; Palm et al., 1989). Cutleaf nightshade (*Solanum strumarium* L.) and jimsonweed (*Datura stramonium* L.) were not adequately controlled by rimsulfuron at the rate of 0.035 or 0.070 kg/ha, respectively (Robinson et al., 1996; Eberlein et al., 1994). Ackley et al. (1996) reported variable control of jimsonweed, but consistently <80%.

Rimsulfuron will also control some annual grasses and sedges. Large crabgrass (*Digitaria sanguinalis* L.), fall panicum (*Panicum dichotomiflorum* Michx.), goosegrass (*Eleusine indica* L.), smooth crabgrass (*Digitaria ischaemum* Schreb.), wild oats (*Avena fatua* L.), proso millet (*Panicum miliaceum* L.), barnyardgrass (*Echinochloa crus-galli* L.), annual sorghum (*Sorghum halapense* L.), green foxtail (*Stellaria viridis* L.), and giant foxtail (*Stellaria faberi* Herrm.) were controlled ≥90% by 0.035 kg/ha.(Eberlein et al., 1994; Mekki and Leroux, 1994; Palm et al., 1989). Marginal to adequate control (76%) of goosegrass was obtained at the same rate (Bewick et al., 1995). Palm et al. (1989) also reported rimsulfuron control of the perennial grasses johnsongrass and quackgrass (*Agropyron repens* L.). At high rates (ai/ha), rimsulfuron will control *Cyperus spp*. (Morales-Payan, 1999; Palm et al., 1989). At lower rates the control of yellow nutsedge (*Cyperus esculentus* L.) is variable. Ackley et al. (1996) showed in a three year study POST applications of 0.035 kg/ha of rimsulfuron controlled yellow nutsedge 51-89%. Findings by Bewick et al. (1995) are in agreement with this, indicating 0.072 kg//ha were required to control yellow nutsedge >90%.

Potato exhibited excellent tolerance to rimsulfuron (Ackley et al., 1996; Robinson et al., 1996; Blackshaw et al., 1995; Eberlein et al, 1994). Rimsulfuron applied between 0.009 to 0.080 kg/ha did not reduce potato yields (Ackley et al, 1996; Robinson et al., 1996; Blackshaw et al., 1995; Eberlein et al, 1994). Visible injury was slightly variable between the studies (0-26% 2 weeks after planting) (Eberlein et al, 1994; Robinson et al., 1996). No reports indicate potato yields will be reduced when rimsulfuron is applied under the rate of 0.070 kg/ha (Ackley et al., 1996; Robinson et al., 1996; Blackshaw et al., 1995; Eberlein et al., 1994). However, visual injury differences have been observed among potato cultivars. Blackshaw et al. (1995) noted that 'Niska' and 'Ranger Russet' had less visual injury than 'Shepody', 'Norchip', or 'Russet Burbank'. Visual injury does not always translate into yield loss. 'Niska' showed little visual injury, but had as many cracked tubers as the more sensitive cultivars 'Shepody', 'Norchip', and 'Russet Burbank' (Blackshaw et al., 1995). 'Fontenot' is a red skinned cultivar grown for fresh market, while 'Atlantic' is a white skinned cultivar for either fresh or processing. Rimsulfuron did not visibly injure 'Fontenot' or 'Atlantic' (injury was ≤ 3% up to 0.070 kg/ha PRE or POST) (Robinson et al., 1996). The cultivar 'Superior' (a short seasoned white table stock cultivar) exhibited up to 26% visual injury from 0.070 kg/ha POST (Ackley et al., 1996). Similar to the study by Blackshaw et al. (1995), the visual injury did not translate into a potato yield reduction (Ackley et al., 1996). A separate study by Porter and Sisson (1994) also indicated 'Superior' was the most sensitive of the six cultivars tested. The levels of sensitivity reported by Porter and Sisson (1994) were not consistent between years (1992 vs.1993). Based on foliar injury, cultivar sensitivity of potato to rimsulfuron has been documented. The sensitivity appears to be partially dependent on environmental conditions, since the level of sensitivity is not consistent (Porter and Sisson, 1994). Regardless of foliar injury, yield and quality parameters (bulk density and cracked potatoes) have been consistent among the cultivars treated with rimsulfuron (Ackley et al., 1996; Robinson et al., 1996; Blackshaw et al., 1995).

As with potatoes, soybean tolerance to rimsulfuron has also been variable among cultivars (Nemat Alla and Hassan, 1996). Three soybeans cultivars, 'Williams', 'Cutler 71', and 'Lyra 71', were treated with 0.005 to 0.025 kg/ha of rimsulfuron POST. As the rate of rimsulfuron applied to the three soybean cultivars increased, the dry wieght decreased. Based on dry weight 'Williams' was more tolerant than 'Cutler 71', which was more tolerant than 'Lyra 71' (Nemat Alla and Hassan , 1996). To reduce the dry weight 10%, 0.013, 0.005, and 0.003 kg/ha of rimsulfuron was needed for 'Williams', 'Cutler 71', and 'Lyra 71', respectively. In the study by Nemat Alla and Hassan (1996) the soybeans were not grown to maturity. Therefore, any possible correlation of rimsulfuron dry weight reduction with yield reductions could not be confirmed. Mekki and Leroux (1994) reported the soybean cultivar tested, at the two trifoliate leaf stage, was very susceptible to rimsulfuron.

Rimsulfuron is labeled for use PRE or POST in processing tomatoes in the United States. The labeled use rate is 0.0175 to 0.035 kg/ha. Different cultivars are used for tomatoes destined for the processing market compared with the fresh market. Bewick et al. (1995) studied the response of tomato to rimsulfuron, using forty nine tomato cultivars. The cultivars selected were either fresh market tomatoes or processing tomatoes. They found there were differences in tomato cultivar response to rimsulfuron. Major differences were observed in the greenhouse, but were not evident in field studies. Processing and fresh market cultivars that were tolerant, moderately tolerant, and susceptible were selected for field studies. All cultivars exhibited general yellowing two weeks after treatment (WAT) and recovered soon after. There were differences in dry weight of the processing and fresh market cultivars. The differences in dry weight were attributed to the morphological differences in the plants (determinate vs. indeterminate). No generalizations could be made on tomato sensitivity to rimsulfuron, based on breeding, or product destination (fresh market vs. processing tomato). From this study, the authors determined tomato sensitivity in the greenhouse did not correspond to field conditions, and each cultivar should be individually tested for sensitivity to rimsulfuron (Bewick et al., 1995).

Halosulfuron is currently labeled for POST use in maize (0.036 kg/ha), grain sorghum (0.036 kg/ha), turfgrass and turfgrass sod (0.035 to0.070 kg/ha), sugar cane and rice (0.018 to0.140 kg/ha), cucumbers (0.0269 to 0.036 kg ai/ha) and woody ornamentals under the trade name Manage®, Permit®, Sandea®, Sempra®, respectively (Servian® outside the United States). If the safener MON 13900 is included with halosulfuron (Battalion®), then it can only be used PRE or pre-plant incoporated (PPI) in maize. All commercially available formulations of halosulfuron are 75 % ai in a wettable granular formulation, except for Battalion®, which is 15% safener and 45% halosulfuron in a wettable granular formulation.

Halosulfuron will provide control of sedges, some broad leaves, and some grasses before emergence. Halosulfuron provides equivalent control of kyllinga (*Cyperus sesquiflorus* L.), purple (*Cyperus rotundus* L.) and yellow nutsedge. In a three year study, the sward dry weight of the sedge kyllinga was controlled 62-96% in a pasture environment with the rate of 0.050 kg/ha (Henskens and Fallow, 1998). Similarly, halosulfuron applied at 0.009 to 0.072 kg/ha initially controlled purple nutsedge 80-100% (Rao and Reddy, 1999; Nishimoto et al., 1997; Webster and Coble, 1997; Hurt and Vencill, 1994). The variation in control can be attributed to the different rates used and different growing conditions. Some of these trials were conducted in established turf, while others were conducted in a maize field and greenhouse. Halosulfuron applied POST between 0.009 to 0.053 kg/ha will provide initial visual control of yellow nutsedge ranging from 79 - 92% (Ackley et al., 1996; Hurt and Vencill, 1994). Ackley et al. (1996)

and Vencill et al. (1995) reported that halosulfuron POST (0.035 and 0.053 kg/ha) reduced yellow nutsedge dry weight 78% and 71%, respectively.

Excellent residual control of purple and yellow nutsedge has been exhibited by halosulfuron. Halosulfuron will control regrowth of purple nutsedge from 7 to 8 WAT (Nishimoto et al., 1997; Vencill et al., 1995). Ackley et al. (1996) reported that regrowth was reduced 100% 7 WAT , while Vencill et al. (1995) reported >95% control 8 WAT. As expected, lower rates of halosulfuron do not provide as long as residual control as higher rates. Five WAT, regrowth of yellow nutsedge was reduced 75 and 83%, from 0.009 and 0.018 kg/ha, respectively (Hurt and Vencill, 1994). One POST application of halosulfuron (0.070 kg/ha) tank mixed with MSMA (2.2 kg/ha) controlled yellow nutsedge shoots 73-79% three months after application (Lowe et al., 2000). In the study by Lowe et al. (2000), halosulfuron applied once/year POST at the rate of 0.070 kg/ha was effective in reducing the tuber population 95% in three years. This herbicide not only exhibits excellent initial control of the sprouted shoots, but also provides control of buds in basal bulbs and tubers inhibiting regrowth (Rao and Reddy, 1999; Nishimoto et al., 1997; Webster and Coble, 1997; Ackley et al., 1996). Either halosulfuron is translocated throughout the nutsedges, effectively causing death of all buds, or the herbicide remains active inhibiting the bud during regrowth. Evidence that halosulfuron is rapidly translocated and remains in the plant is supported by Khan and Molin (2001).

In a study by Sprague et al. (1997), halosulfuron was applied PRE at the rate of 0.084 kg/ha and weed control was visually rated 60 days after treatment. Baird et al. (1995), using 0.071 kg/ha of halosulfuron POST, evaluated weed control, and reported findings similar to that of Sprague et al. (1997). In the study conducted by Sprague et al. (1997) halosulfuron provided superior control compared to atrazine. However, the findings of Sprague et al. (1997) and Baird et al. (1996) have not been confirmed in all studies conducted on halosulfuron.

Sprague et al. (1997) found halosulfuron provided 79-94% control of velvetleaf (*Abutilon theophrasti* Medik.). POST applications between 0.0347 to 0.0526 kg/ha of halosulfuron will control velvetleaf 96-98% (Fausey and Renner, 1996; Wax et al.,1996). Sprague et al. (1997) reported >90% control of common lambsquarters (*Chenopodium album* L.), but all early postemergence (EPOST) and POST applications of halosulfuron have provided considerably less control (60 and 23%, respectively) (Owens et al., 1997; Wax et al., 1996; Hart and Maxwell, 1995; Majek, 1994). The rates of EPOST and POST applications were much lower 0.01 to 0.035 kg/ha, compared to the rate (0.071 kg/ha) used by Sprague et al. (1997). Halosulfuron has provided excellent control of Pennsylvania smartweed (*Polygonum pensylanicum* L.) when applied both PRE and POST (>75% control) (Fausey and Renner, 1996;Wax et al., 1996; Lueschen and Getting, 1995). The height of Pennsylvania smartweed appears to be correlated to control by halosulfuron. As height of Pennsylvania smartweed increases beyond 10 inches control decreases (Lueschen and Getting, 1995). Control of both giant and common ragweed has generally been favorable when using halosulfuron, but control has varied between 48% and 98% depending on the study location (Hart and Maxwell, 1995; Baird et al., 1996; Lueschen and Getting, 1995). When halosulfuron was applied PRE or POST to common cocklebur, control was 87-98% (Sprague et al., 1997; Lueschen and Getting, 1995).

Height of common cocklebur did not appear to be as significant a factor in control by halosulfuron as compared to Pennsylvania smartweed. PRE and POST control of jimsonweed has been excellent (90-98%), but POST applications were made when jimsonweeds were 2 inches tall (Sprague et al., 1997; Baird et al., 1996). Majek (1994) indicated common purslane and hairy galinsoga were effectively controlled by POST applications.

Some pigweeds (*Amaranthus* species) may not be controlled by halosulfuron. Halosulfuron controlled smooth pigweed 98% with POST applications of 0.0347 kg/ha (Hart et al., 1995). Bottenberg et al. (1997) reported halosulfuron applied PRE at 0.0526 kg/ha only controlled pigweeds 50%. In Bottenburgs' study no mention was given to the pigweed (*Amaranthus*) species, or history of ALS resistant pigweeds in the area the study was conducted. Krausz and Kapusta (1997) reported halosulfuron applied POST to waterhemp (*Amaranthus tuberculatus* Moq.) at 0.0347 kg/ha did not provide any control of this weed. An earlier report by Krausz et al. (1995) indicated 50% control of waterhemp and 99% control of redroot pigweed.

Wild carrot control is marginal with halosulfuron. Halosulfuron applied PRE at 0.084 kg/ha did not significantly reduce the number of plants emerged compared to the control, but fresh weight was reduced 86% (Stachler and Kells, 1997). When halosulfuron is applied POST at 0.041 kg/ha, control of wild carrot weight is reduced to 67% (Stachler and Kells, 1997). In field studies where maize was being grown, halosulfuron applied POST at 0.041 kg/ha controlled wild carrot 79-95% (based on visual ratings)(Stachler and Kells, 1997). Competition from the maize likely increased the control of wild carrot in the field setting. Halosulfuron will provide some control of *Striga spp.* when applied as a drench seed treatment (Abayo et al., 1998). Halosulfuron would not significantly control burcucumber, white clover, or perennial ryegrass (Messersmith et al., 1999; Henskens and Fallow, 1998).

When halosulfuron is applied before giant foxtail reaches three leaves (PRE or EPOST) 99% control has been reported with lower rates, but this control was reduced to 50% when applied POST (0.0347 kg/ha)(Harvey et al., 1995a,b,c). Halosulfuron applied at 0.0526 kg/ha to green, giant, and yellow foxtail less than 9 inches tall resulted in 40% control (Baird et al., 1996). Timing or physiological age seems to be an important factor in foxtail control, when using halosulfuron. Large crabgrass, barnyard grass , wooly cupgrass (*Eriochloa villosa*), fall panicum, and shattercane (*Sorghum bicolor*) were treated with halosulfuron before they reached 8 inches, with the exception of barnyard grass, which was 11 inches tall, and none were effectively controlled by 0.0526 kg/ha of halosulfuron applied POST (Baird et al., 1996).

Halosulfuron is an excellent sedge herbicide, providing excellent control under various application rates, environments and timings. Halosulfuron control of broadleaf weeds is not as robust compared to sedges. The spectrum of broad leaf control increases as the rate of herbicide applied increases. This effect is evident in common lambsquarter control. The age of the plant in terms of tolerance to halosulfuron was significant for common lambsquarter, Pennsylvania smartweed and the foxtails. The species was a significant factor in halosulfuron tolerance for the *Amaranthus spp.* Redroot pigweed and smooth pigweed were consistently controlled by halosulfuron, but waterhemp control varied between 0 to 50%. It is hard to determine the contribution of environment to the variation reported for *Amaranthus spp*. control.

The genetic line of the crop has been shown to influence tolerance to halosulfuron. Tolerance of six sweet corn hybrids to halosulfuron were tested under field conditions. POST applications of 0.0716 kg/ha reduced the yield of all hybrids except 'Excellency'(Van Wychen et al., 1997). Interestingly the vigor was only reduced in two of the six cultivars. The genetic line of maize appears to be more consistent in terms of tolerance to halosulfuron. Baird et al. (1996) conducted a study to determine the tolerance of 15 maize cultivars [4 hybrid, 10 inbred, and 1 imidazolinone resistant (IR) cultivar] to halosulfuron. They found that PRE applications of 0.105 kg/ha of halosulfuron reduced the vigor of all maize 10 to 50%, except the IR. Furthermore, vigor in the majority of the cultivars were reduced >20% (Baird et al,.1996). When halosulfuron was applied POST at 0.0705 kg/ha maize vigor was reduced 0-30%, with the majority of the cultivars being reduced 10% (Baird et al., 1996). It appears that all cultivars respond similarly to halosulfuron and it is the application timing that is crucial to the plant's tolerance to the herbicide. Differences in cultivar response of sugarcane to halosulfuron have been negligible. In a study conducted in Mauritius, all but one cultivar tolerated halosulfuron applied POST up to 0.080 kg ai/ha (Barbe et al., 1997). The authors did not indicate if the yield of the intolerant cultivar was reduced. Other reports have indicated no yield reductions or visual injury from POST halosulfuron applications on sugar cane (Leibbrandt, 1997; Mascarenhas et al., 1995). Based on one report, evidence of sugarcane cultivar (genetic line) influencing halosulfuron tolerance exists. However this same cultivar was not injured by halosulfuron in other trials, so environmental conditions may also influence sugarcane tolerance of halsoulfuron.

Turf has exhibited excellent tolerance to halosulfuron. Studies using *Paspalum vaginatum*, *Cynodon dactylon*, *Poa pratensis*, *Agrostis esculentus*, *Lolium perenne* have shown these lines of turf to be tolerant to halosulfuron POST (Blum et al., 2000; Johnson and Duncan, 1997; Nishimoto et al., 1997; Fry et al., 1995; Jackson et al., 1993). Fry et al. (1995), however, did not find the perennial rye grass (*Lolium perenne*) to be tolerant to halosulfuron POST at rates much lower than those reported by Jackson et al. (1993). The unacceptable damage described by Fry et al. (1995) was in visual coloring and not plant mortality or height reduction. This discrepancy may be based on the rating system, cultivar, or environmental conditions.

Azalea (*Rhododendron indicum*), redtip photinia (*Photinia fraseri*), green liriope (*Liriope muscari*), white petunias (*Petunia hybrida*), red petunias (*P. hybrida* cv. Red Madness), lavender petunias (*P. hybrida* cv. Orchid Madness), celosia (*Celosia plumosa*), vinica (*Vinca roseus*), African marigolds (*Tagetas erecta*), bronze-leaved begonias (*Begonia semperflorens*), and purple salvia (*Salvia splendens*) exhibited acceptable tolerance to halosulfuron (Hurt and Vencill, 1994). Some similar species were not as tolerant to halosulfuron. For instance, french marigold (*T. Patula*), red salvia (*S. splendens* cv Red Hot Sally), and green-leaved begonia (*B. caculatta*) were injured >10% by the same rate of halosulfuron. Azaleas were reportedly injured (21%) by high (0.28 kg/ha) PRE rates of halosulfuron (Derr et al., 1994). The different reports of azalea tolerance at least indicate a rate effect of halosulfuron if not a cultivar effect. Cotoneaster

(*Cotoneaster dammeri* cv coral beauty) and crape myrtle (*Lagerstroemia indica* cv natchez) were injured 53 and 24%, respectively by 0.28 kg/ha of halosulfuron applied PRE (Derr et al., 1994). As with the weeds, it appears that crop tolerance to halosulfuron is dependent on genetic background, environmental conditions, and rate.

Species and cultivar specific tolerance of SU have been well documented in maize, oats, turf, barley, soybeans, and tomatoes (Newson and Shaw, 1992; Stall and Bewick, 1992; Green and Ulrich, 1993; Bewick et al., 1995; Williams and Harvey, 1996; Green, 1998; Mathiassen et al., 1999; Urban and Adamczewski, 1999). Differential tolerance in weed species to SU have also been reported. Nightshade (*Solanum spp.*) is not equally sensitive to rimsulfuron (Bewick et al., 1991; Ackley et al., 1999). Augustin et al. (1998) reported differential responses of cheat (*Bromus spp.*) to sulfosulfuron. Three *Cirsium spp.* were found to exhibit differential tolerance to metsulfuron, chlorsulfuron, and triasulfuron (Solymosi and Nagy, 1998). Sterling and Jochem (1995) reported differential tolerance of two locoweed species to metsulfuron. Selecting 94 cultivars of field and sweet corn, Green and Ulrich (1993) tested the cultivar sensitivity of primisulfuron, thifensulfuron, and nicosulfuron in maize. Their study indicated that tolerance to the SU was imparted by a single recessive gene, since at least one parent must be tolerant to produce tolerant offspring. Stall and Bewick (1992) also indicated the level of sensitivity was influenced by genetic lines, since Sh_2 lines were the most sensitive to nicosulfuron.

It is clear genetics influence the tolerance of plants to SU. What is not clear is which gene is responsible for tolerance and what function is being expressed by this gene in each species. Koeppe and Brown (1995) reported the tolerance of crop plants to the SU is based on metabolic breakdown of the herbicide. Evidence exists that metabolism is responsible for tolerance in all crop plants. Furthermore, weed species have exhibited several mechanisms of tolerance to the SU. Mechanisms of plant selectivity to SU have paralleled other herbicides. The primary mechanism of tolerance in crops and some weed species is metabolism of the SU into non-phytotoxic metabolites. Insensitive ALS enzymes have been reported in both crops and weeds. Differential uptake and translocation is not common, but has been observed. Environmental conditions can affect the activity of SU, but this is not consistently a factor that influences plant selectivity.

Target Site Sensitivity

Target site resistance in herbicides was first reported in 1968 with a member of the triazine family (Holt et al., 1993). Species resistant to photosystem II inhibitors were deemed less "fit" than susceptible species (Radosevich et al., 1997). Meaning, that in the absence of the selection pressure from the herbicide, the species would no longer dominate the weed spectrum. Species that evolved resistance to ALS inhibitors have demonstrated fitness similar to susceptible species. Resistance in smooth pigweed, Canadian thistle (*Cirsium arvense* L.), sunflower (*Helianthus annuus* L.), kochia, ryegrass (*Lolium rigidum* L.), Russian thistle (*Salsola iberica* Sennen), sowthistle (*Sonchus oleraceus* L.), and black medic (*Stellaria media*) is reportedly due to altered ALS (Baumgartner et al., 1999; Manley et al., 1999; Boutsalis and Powles, 1995; Kwon and Penner, 1995; Christopher et al., 1992; Saari et al., 1992; Devine et al., 1991). The activity (Km, Vmax, and specific activity) of the ALS in the resistant and susceptible biotypes of sunflower, kochia, ryegrass, sowthistle, and Russian thistle was similar (Baumgartner et al., 1999; Boutsalis and Powles, 1995; Kwon and Penner, 1995; Saari et al., 1992). It would seem that these resistant biotypes would grow and reproduce normally. Fitness of the black medic population was tested by vegetative growth, seed germination, and seed weight (O'Donovan et al., 1995). The resistant biotype was equal or better than the susceptible biotype in all growth parameters tested.

Herbicides in the aryloxyphenoxy propionate and cyclohaxanedione family inhibit the enzyme acetyl-CoA carboxylase (ACCase). Most broad leaves are resistant to these herbicides, because they contain both sensitive and insensitive ACCase isozymes. Plants not containing the insensitive ACCase are sensitive to aryloxyphenoxy propionate and cyclohaxanedione herbicides (Christopher and Holtum, 2000). A *Lolium multiflorum* biotype in France that contains a susceptible and resistant isozyme has been documented (Prado et al, 2000). Biotypes of maize that contain ACCase insensitive to either aryloxyphenoxy propionate and/or cyclohaxanedione herbicides have also been well documented and commercially exploited (Marshall et al., 1992). Their research has suggested through heterozygous crosses, that at least two ACCase isozymes (tolerant and sensitive) are being expressed (Marshall et al., 1992).

Soybeans containing naturally altered ALS, tolerant to ALS inhibiting herbicides, have been documented and commercially exploited as well. Target site mutations that render the plant resistant to the herbicide have occurred in both weeds and crops. In both the weed and crop plants, no adverse response was exhibited as a result of the target site mutation. Therefore it would seem possible for tomato to be tolerant to SU through altered ALS or an insensitive isozyme and still produce normal yields.

Acetolactate Synthase

ALS was discovered long before the herbicidal properties of the SU, imidazolinones, or triazolopyrimidines. One of the first descriptions of acetolactate synthase was by Juni (1952). Since the discovery of ALS the enzyme has been found in higher plants, bacteria, and yeast. In bacteria and yeast isozymes have been confirmed. In higher plants, they are not as common and the enzyme is located in the plastids or chloroplasts. While the enzyme is constitutively expressed the levels of expression are not static. ALS levels in etiolated shoots (containing only plastids) of barley, were higher than "green" shoots (Durner and Boger, 1988). Evidence has been reported indicating expression of ALS could be regulated by light and tissue development. Durner and Boger (1988) did not indicate any differences in enzymatic activity between the plastids and chloroplasts. The increased levels of enzyme could have been a result of isozyme expression as Durner and Boger (1988) indicated.

ALS Activity

This enzyme catalyzes the formation of S-acetolactate by condensation of two molecules of pyruvate. The condensation of pyruvate with a-ketobutyrate yields a-aceto-a-hydroxybutyrate. S-acetolactate is a precursor in the synthesis of the branch chain amino acids valine and leucine, while a-aceto-a-hydroxybutyrate is the precursor for the branch chain amino acid isoleucine. ALS has a requirement of flavin adenine dinucleotide (FAD), thiamine pyrophosphate (TPP), and a divalent metal cofactor being present for activity. FAD is typically used in oxidation/reduction reactions. It is not understood why ALS has a requirement for FAD, when no net change in the ALS charge can be observed during the reactions. TPP is used in binding of the first pyruvate to the ALS. A divalent cation is needed as a co-factor in the binding of TPP. Not all divalent cations are sufficient or equally efficient as co-factors. The specific activity of ALS in peas and beans is reportedly higher in the presence of Mg^{+2} followed by Mn^{+2}(Davies, 1964; Satyanarayana and Radhakrishnan, 1962). Activity of bean ALS was inhibited in the presence of Ag^{+2} and Hg^{+2} (Satyanarayana and Radhakrishnan, 1962).

Kinetics are used in enzymology to study the rate at which a given reaction will proceed. In the synthesis of valine and leucine, ALS reacts twice with pyruvate. A reaction that involves two substrates forming one product is typically termed second order kinetics. In first order kinetics, the rate of the reaction is dependent on the concentration of only one reactant (Roberts, 1977a,b). ALS is still reported to follow first order kinetics (Chipman et al., 1990), presumably because it is reacting with the same substrate twice. The Km is a parameter used in kinetic studies, which describes the concentration of substrate required to reach ½ of the maximal velocity (Vmax) of a reaction. This parameter is used for comparing affinity of enzymes. The Km of barley ALS was 14.3 mM (Miflin,1969) and 5.5 mM by (Durner and Boger, 1988). The Km of pea ALS was 3.2 and 43 mM, depending on the "isozyme" (Davies, 1964). Phaseolus ALS had a Km of 31 mM (Satyanarayana and Radhakrishnan, 1962). Lettuce ALS has been reported to have a Km of 7.3 and 11.1 for lines resistant and susceptible, respectively, to ALS inhibiting herbicides (Eberlein et al., 1999). Singh et al. (1988b) reported maize ALS had a Km of 5 mM. The Km of ALS in soybean ranged between 63-74 mM, depending on the cultivar used (Nemat Alla and Hassan, 1996). ALS III from *E. coli* exhibited first order kinetics in the reaction forming acetolactate, with a Km of 6mM. ALS III has a similar Km to the majority of higher plants reported.

ALS Structure and Isozymes

Differing size of enzymes, pH optima, sensitivities to feed back inhibition, different coding genes, and differing Km have been used as indicators of isozymes. No single criterion to identify isozymes is consistent for all species. In bacteria, six isozymes of ALS have been identified and reported (Schloss, 1990). The enzymes are made up of two subunits, a large (59 kDa) and small (9.5 kDa), in an $\alpha_2\beta_2$ configuration (Schloss, 1994). The sensitivity of bacteria's isozymes to inhibitors varies. ALS I is insensitive to sulfonyl urea inhibition. ALS II and III are sensitive to inhibition by SU, but ALS II is insensitive to feed back inhibition by all three branch-chain amino acids. These isozymes are of similar molecular weights, but the genetic coding regions and the biological activity are not.

Evidence of isozymes in higher plants have been reported in tobacco, barley, and maize (Durner and Boger, 1988; Lee et al., 1988; Singh et al., 1988). Subramanian and Gerwick (1989) reported there are two isoforms of ALS present in barely, with only one enzyme being moderately stable.

Valine

2 Pyruvate+ $\xrightarrow{\text{TPP}}$ α-Acetolactate + NADPH+H$^{\oplus}$ → + H_20 →

→ α-Ketoisovalerate + Glutamate → **Valine** + α-Keto-glutarate

Leucine

α-Ketoisovalerate + → α-Isopropylmalate → β-Isopropylmalate →

→ β-Isopropylmalate + NAD⊕ → NADH + α-Ketoisocapronate →

→ α-Ketoisocapronate +Glutamate → a-Keto-glutarate + **Leucine**

Isoleucine

α-Keto-butyrate + Pyruvate → + NADPH → NADP⊕ + H_2O → + Glutamate →

α-Ketoglutarate + **Isoleucine**

Durner and Boger (1988) indicated that the two molecular weights (440 and 200 kDa) of ALS they found in barley were a results of different "polymeric forms of a basic unit". The subunit in this study was reported to be 58 kDa, which is similar to the subunit size reported in bacteria (Schloss, 1990). In tobacco, Lee et al. (1988) indicated that there were two distinct genes on separate loci encoding for ALS, and one of the genes was responsible for plant tolerance to SU. Using black Mexican sweet corn cells, Singh et al.(1988) reported on two forms of ALS that had different molecular weights (193 vs. 55 kDa). They determined the two forms of ALS were isozymes, because of the different pH optimums, Km, and feed back inhibition exhibited. The most convincing data of ALS isozymes came from Lee et al. (1988) and Singh et al. (1988). In their work, evidence of isozymes came from not only physical differences in ALS (molecular weight and genetic coding regions), but in biological differences that related to activity of the enzyme.

Enzyme Kinetics

Temperature, pH, time, substrate, and enzyme concentration influence the rate of enzyme activity. Enzymatic reactions increase in their rates as temperature increases up to an optimal point. There is a concurrent increase in the excitation of the substrate molecules from the collision with each other. These excited molecules contain energy that is required for the reaction to proceed to a product. The energy required to proceed to a product is described as activation energy (Roberts, 1977). Temperature influences the activation energy, and ultimately the rate of the reaction. The pH in which a reaction occurs will influence the rate of this reaction. Substrates and enzymes contain many different functional groups and amino acids that can be influenced through ionization. Therefore, pH can influence enzymatic reactions equally in many different ways (Engel, 1996; Roberts, 1977).

Time and enzyme concentration are two important factors of an assay that influence the Km and Vmax of an enzyme. These two parameters are used to define the rectangular hyperbolic model described by Michaelis-Menton equation (Tipton, 1995). This equation uses three assumptions that must be valid for an accurate estimation of the Km and Vmax. The first assumption is the reaction proceeds in the forward direction. Another assumption is the enzyme substrate complexes are in the steady state. As time continues it is possible that the reaction may proceed in a reverse direction, i.e. product to substrate (Tipton, 1995). Not all enzymes are definitely stable over time. Therefore the assay time in a kinetic experiment should be conducted so the activity is linear with relation to time (Engel, 1996; Tipton, 1995). Once a suitable incubation time is established for the assay, the concentration of an enzyme used needs to be determined. The last assumption is the enzyme substrate complexes do not significantly reduce the substrate levels. High enzyme concentrations may lead to complexes that co-operatively reduce enzyme levels faster than at ½ the concentration (Tipton, 1995). Theoretically, doubling the concentration of the enzyme added should double the product formed. If the product doubles, then the enzyme concentration is in the linear portion of activity (Engel, 1996; Tipton, 1995). The enzyme concentration should be in the linear portion of the activity curve, but high enough to accurately measure the reaction over time (Engel, 1996). Once the conditions of the assay are established, the kinetics of the enzyme can be investigated by assaying activity at varying substrate levels and inhibitor levels.

Proper assay conditions are only part of accurately determining the Km and Vmax. Traditionally a Lineweaver-Burk plot (here after referred to as a double reciprocal plot) was used to characterize enzymatic activity, and estimate the Km and Vmax. There are inherent problems with using a double reciprocal plot or a Eadie-Hofstee plot (V plotted against V/S) to estimate the Km or Vmax (Henderson, 1995). In these plots the variation is not homogenous (Henderson, 1995). Direct linear plots (V is plotted against -S) or least squares are the preferred methods to estimate the Km or Vmax (Henderson, 1995). The least square method must be appropriately weighted for accurate estimated of the Km and Vmax (Henderson, 1995). The double reciprocal plots are still useful for checking outlying data and validating assumptions of Michaelis-Menton. Henderson (1995) and others recommend using the least square to determine inhibition constants.

ALS Inhibition

Inhibitors of enzymes are classified as reversible or irreversible (Tipton, 1995; Roberts, 1977). All of the inhibitors of ALS from higher plants (SUs, imidazolinones, and triazolopyrimidines) reportedly inhibit the ALS both reversibly and irreversibly. The irreversible inhibition is time and herbicide dependent (Hawkes, 1989). When ALS is inhibited after the one pyruvate is bound to the ALS, then it is sensitive to the oxygenase reaction (Abell and Schloss, 1993). Imidazolinones bind tighter than SUs to ALS after one pyruvate is bound with the enzyme (Schloss et al., 1988). Most of the ALS should be bound to pyruvate, since initial binding occurs at low concentrations (Schloss, 1994). So the oxygenase, and therefore the irreversible loss of activity, won't occur at high pyruvate concentrations (Tse and Schloss, 1993). The initial loss of activity from SU and imidazolinones is through reversible inhibition.

There are several ways an inhibitor interacts with the enzyme and substrate. If the inhibitor competes with the substrate for the substrate binding site on the enzyme, it is said to be a competitive inhibitor. An inhibitor that only binds to the enzyme after the substrate is bound is said to be an uncompetitive inhibitor. Non-competitive and mixed inhibitors bind to both the enzyme and the enzyme-substrate complex (Tipton, 1995; Roberts, 1977). The nature of the inhibition can be determined by plotting the activity (product formation) against the substrate concentration as a double reciprocal plot. A competitive inhibitor is graphically represented by several activity lines intersecting at the y axis. Non-competitive inhibitors are represented by the activity curves intersecting left of the y axis on the x axis. Uncompetitive inhibitors are represented by a series of parallel curves. Mixed inhibitors are rare, but are represented by intersecting curves to the left and above the y and x axis, respectively. Double reciprocal, Eadie-Hofstee, and Cornish-Bowden plots can be used to plot inhibition data. The direct linear plot is not recommended to use for graphing inhibition data (Henderson, 1995).

All inhibitors of ALS tested have been shown to be non-competitive. Other evidence supporting that SU, imidazolinones, and triazolopyrimidines inhibit in a similar manner was provided by Butler and Siehl (1992). Their research indicates synthetic inhibitors of ALS compete with each other for the same binding site. All ALS inhibitors initially exhibit reversible binding at the same site, but irreversible binding occurs overtime, when one pyruvate is bound to the enzyme with the inhibitor. When this occurs, there are differences between the SU and imidazolinones in affinity to ALS and therefore differences in irreversible inhibition (Schloss, 1994; Muhitch et al., 1988). Since the size of the inhibitors that apparently bind to the same site differ, it has been proposed that the binding site on ALS is large (Butler and Siehl, 1992). This would explain the differences in affinity and the reversible versus irreversible inhibition occurring on the same binding site.

Uptake and Translocation

Some plants exhibit limited uptake and/or translocation of a herbicide applied to it. The two events (uptake and translocation) are not necessarily mutual events. Differential tolerance of two alfalfa (*Medicago sativa*) cultivars toward terbacil was investigated by Anderson et al. (1995). They determined that the two cultivars absorbed different quantities of the herbicide (sensitive cultivar absorbing more), but they translocated the absorbed herbicide in equal proportions (Anderson et al., 1995). Two cultivars of soybean exhibiting differential tolerance to paraquat were shown to absorb equal amounts of the herbicide, but translocation into interveinal areas was much higher in the susceptible cultivar (Kim and Hatzios, 1993). Isoxaben tolerance in the ornamental species *Euonymous* and *Ajuga* was affected by uptake and translocation (Salihu et al., 1998). Uptake, translocation, and metabolism were occurring in all three species, but the most tolerant species exhibited moderate uptake and translocation (Salihu et al., 1998). Kentucky bluegrass (*Poa pratensis* 'Plush') tolerance to quinclorac was investigated by comparing to a susceptible species *Digitaria ciliaris* (Chism et al., 1991). Uptake of *D. ciliaris* was more rapid compared to bluegrass. Furthermore, the bluegrass exhibited a greater distribution of the herbicide than *D. ciliaris* and exhibited a unique mechanism of exuding the herbicide from the roots (17%).

Plant tolerance to herbicides can be achieved through multiple mechanisms i.e. limited uptake and/or translocation combined with metabolism. Fluroxypyr activity was studied in two susceptible wild buckwheat (*Polygonum convolvulus*) and field bindweed (*Convolvulus arvensis*) and two tolerant species thistle (*Cirsium album*) and Canadian thistle. All species absorbed equal amounts of herbicide (MacDonald et al., 1994). Fluroxypyr tolerance of thistle and Canadian thistle was attributed to decreased translocation and increased metabolism. Cotton has been shown to metabolize and sequester (not translocate) pendimethalin (Shaner et al., 1998). A similar situation has also been reported in rice and lettuce. The safener fenclorim reduced the uptake and increased the metabolism of pretilachlor in rice (Han and Hatzios, 1991). Reiners et al. (1988) reported increased translocation and decreased metabolism of thiobencarb in a susceptible cultivar of lettuce. *Viola arvensis* has been shown to exhibit increased tolerance to terbacil with increasing physiological age from 3 leaf to 12 leaf (Doohan et al., 1992). Three leaf *Viola* absorbed and translocated more terbacil than 12 leaf plants. In addition, 12 leaf plants showed higher levels of terbacil metabolites compared to 3 leaf plants, indicating increased metabolism.

There are few examples of limited uptake and/or translocation of SU herbicides in higher plants. Mechanisms of differential tolerance of cocklebur, thistle and sicklepod

(*Cassia obtusifolia*) to prosulfuron was investigated (Ma et al., 1995). Uptake was higher in thistle and sicklepod, but translocation was lower in these species. Canadian thistle appears to tolerate other herbicides besides fluroxypyr through decreased translocation. Decreased translocation of chlorsulfuron, metsulfuron, and triasulfuron was implied as one of the mechanism of resistance in two varieties of Canadian thistle, which were reported to have thicker wax layers and less stomata on the leaf surface (Solymosi and Nagy, 1998). Decreased translocation of thifensulfuron in soybeans is observed when this herbicide is applied with bentazon (Lycan and Hart, 1999). Decreased translocation concurrently reduced soybean injury from thifensulfuron without affecting the rate of metabolism (Lycan and Hart, 1999). Reduced translocation of bensulfuron in rice reportedly aides in tolerance (Kwon and Pyon, 1993). Reduced SU uptake was reported as the mechanism of tolerance for two *Ipomoea* species. These species absorbed approximately 1% of the applied chlorimuron (Moseley et al., 1993). Chlorimuron foliar uptake in the sensitive species Florida beggarweed, common cocklebur, sicklepod, and yellow nutsedge was 56, 43, 85 and 11%, respectively (Wilcut et al., 1989; Reddy and Bendixen, 1988). It is apparent that reduced uptake of chlorimuron aids in the selectivity of soybean cultivar 'W-20'. The cultivar 'W-20' absorbed 33% less radioactivity while metabolizing chlorimuron slower than a sensitive and tolerant soybean cultivar (Mosely et al., 1993). Sugar beet absorbed less than 6% of applied triflusulfuron after 20 hours, but selectivity in sugar beets is reportedly through increased metabolism since sensitive species absorbed equal amounts (Wittenbach et al., 1994).

Xenobiotic Detoxification in Plants

Plants contain enzymes utilized in secondary metabolism and/or to maintain homeostasis, however some of these enzymes [cytochrome P-450's (CYP450) and glutathione-S-transferases(GST)] also detoxify xenobiotics (McGonigle et al., 1997; Schuler, 1996). Herbicides are xenobiotics, which are transformed through oxidative, reductive, hydrolysis, or conjugation processes (Hatzios, 1994).

The most common initial transformation of herbicides in plants is oxidation or hydrolysis (Hatzios, 1994). Almost all of the oxidations (N & O-dealkylation, N-deamination, aromatic hydroxylation, thioether oxidation, N-oxidation, and β oxidation) of xenobiotic's reported in plants are catalyzed by the CYP450s, which are a gene super family (Schuler, 1996; Hatzios, 1994). There are over 450 CYP450s that are structurally and functionally diverse (Schuler, 1996). Only a portion of the CYP450s have xenobiotic detoxifying properties, those CYP450s that detoxify xenobiotics are believed to function primarily for catabolizing endogenous substrates (Schuler, 1996). Evidence of multi-functional CYP450s is provided by a *trans*-cinnamic acid hydroxylase that will metabolize *trans*-cinnamic acid and chlortoluron. However, the metabolism of the endogenous *trans*-cinnamic acid is 21,214 times faster than chlortoluron (Pierrel et al., 1994). Characterization of CYP450s is difficult, because of their inherently low levels in plants and their instability once extracted (Schuler, 1996). The levels of CYP450 expression have been elevated, by treating tubers/seeds or plants with manganese, ethanol, phenobarbital, aminopyrine, naphthalic anhydride, and other herbicides (Mougin et al., 1991; Reichhart et al., 1980). It seems that CYP450s responsible for herbicide transformations and detoxification are induced by different stimuli, than CYP450s known to be involved in biosynthesis (Moreland et al., 1993a b; Zimmerlin and Durst, 1992). This increases the difficulty of characterizing a CYP450.

Hydrolysis metabolism of xenobiotics is also catalyzed enzymatically, but the enzymes responsible for this process are in the CYP450 family. Hydrolysis occurs in xenobiotics containing a ester, amide, or nitrile group. The enzyme responsible for hydrolysis of xenobiotic esters in plants has not been identified, but the transformation was observed *in vitro*. Hydrolysis of the amide propanil is catalyzed by aryl acylamidase (Hoagland and Graf, 1972). Enzymes that catalyze the metabolism of nitrile herbicides have not been discovered, but the process does occur (Beynon et al., 1972). Reduction of select herbicides do occur in some plants and is believed to be catalyzed enzymatically, however it is not common (Hatzios, 1994).

The conjugation of herbicide metabolites usually follows oxidative, reductive, or hydrolytic transformations (Hatzios, 1994). In conjugation, a xenobiotic is transformed to a higher molecular weight compound by combining with a sugar, amino acid, or glutathione (Hatzios, 1994). Xenobiotics that conjugate to a sugar usually contain a phenolic, carboxylic acid, or N-arylamine moiety (Hatzios, 1994). The addition of the sugar moiety is controlled enzymatically. UDP-glucosyl transferases have been described in several crops (Schmitt et al., 1985; Frear et al., 1983; Lamoureux et al., 1971).

Not many herbicides are known to be detoxified through conjugation with endogenous amino acids. When chlorpropham or chlorfenprop-methyl is conjugated with cysteine they are metabolically inactive (Collet and Pont, 1978; Still and Rusness, 1977).

Other examples of herbicides conjugating with amino acids have been reported, but the resulting compounds are still biologically active. Therefore these reactions are not detoxifying.

When herbicides are conjugated, most frequently it is with glutathione or homoglutathione as in legumes (Hatzios, 1994). When herbicides or herbicide metabolites are conjugated with glutathione it is usually under the control of glutathione-S-transferases [GST's (E.C. 2.5.1.18)] (Hatzios, 1994). While GST are enzymes, not all herbicide transformations involving glutathione occur enzymatically. Levitt and Penner (1979) showed that EPTC and some acetanilide herbicides conjugate with glutathione non-enzymatically. Unlike the CYP450s, the GSTs are present in all parts of the plant throughout its life cycle (McGonigle et al., 1997). The GSTs are similar to the CYP450's in the control of the induction. The levels of GST can be up-regulated by treatment with exogenous and endogenous compounds (McGonigle et al., 1997). The stimuli responsible for increasing GST levels are different than inducers of CYP450. The GSTs are induced by different plant hormones, polyethylene glycol, KCl, canavine, 2,4-D, and heavy metals (McGonigle et al., 1997).

All of the 23 SU commercially available have been shown to be transformed in plants. In some cases, the contribution of herbicide transformation to plant selectivity is questionable. As indicated above, thifensulfuron is metabolized in soybean, but soybean is still intolerant of this herbicide. When bentazon is applied with thifensulfuron, the uptake of thifensulfuron is reduced while the rate of metabolism remains the same . Therefore, the overall tolerance of soybeans to thifensulfuron is increased (Lycan and

Hart, 1999). Transformation of the SU is occurring, but the importance of enzymatic metabolism to imparting whole plant tolerance is not clear in all herbicide, species, and combinations. Koeppe and Brown (1995) indicated the selectivity of SU in crop plants is achieved through metabolic transformation of these herbicides, and not a function of chemical hydrolysis. Koeppe and Brown (1995) go on to indicate the pathways of transformation are different for each herbicide and plant species.

SU are unique in the multiple routes of metabolic transformation that occur for one herbicide in a species. O-demethylation and hydroxylation are usually the initial steps of transformation reported in plants. After these two initial transformations the compound are typically conjugated, followed by bridge contractions and/or cleavage (Roberts et al., 1998). For a few SU conjugation with glutathione is the initial step of transformation.

O-demethylation is the primary step of transformation for the herbicides amidosulfuron, azimsulfuron, bensulfuron, cinosulfuron, flupyrsulfuron, halosulfuron, imazosulfuron, prosulfuron, and pyrazosulfuron (Roberts et al., 1998; Koeppe et al., 1998; Dubelman et al., 1997; Frear and Swanson, 1996; Yamamoto et al., 1996; Shirakura et al., 1995; Kamizono et al., 1994). Chlorimuron, chlorsulfuron, halosulfuron (in maize), metsulfuron, nicosulfuron, primisulfuron, pyrasulfuron, rimsulfuron, sulfometuron, triasulfuron, and tribenuron have been shown to undergo hydroxylation on either ring (phenyl or pyrimidine/triazine) in treated plants (Koeppe et al., 2000; Roberts et al., 1998; Dubelman et al., 1997; Frear and Swanson, 1996; Yamamoto et al., 1996; Anderson and Swain, 1992; Lamoureux and Rusness, 1992; Fonne-Pfister et al., 1990; Hutchinson et al., 1984; Sweetser et al., 1982).

The transformations observed in treated plants can indicate the mechanisms and regulatory factors at work. Not all transformations in species tolerant to a given SU are identical. Chlorsulfuron will undergo bridge contraction and hydroxylation of the phenyl ring followed by carbohydrate conjugation in tolerant monocots (Sweetser et al., 1982). Tolerant broad leaves metabolize this herbicide through hydroxylation and conjugation with a carbohydrate (Hutchinson et al., 1984). Flupyrsulfuron is conjugated with glutathione in wheat while, *Phalaris minor* metabolizes this herbicide through O-demethylation on the pyrimidinyl ring (Koeppe et al., 1998; Barefoot and Christensen, 1995). Halosulfuron is metabolized by O-demethylation and hydroxylation on the pyrimidine ring in wheat and maize, respectively (Dubelman et al., 1997). Wheat and sorghum metabolize prosulfuron on different rings through different mechanisms (Frear and Swanson, 1996). Primisulfuron is metabolized through hydroxylation of the pyrimidine phenyl ring in maize (Fonne-Pfister et al., 1990). This indicates that unique mechanisms of breakdown for a single SU exist for each species. Evidence that metabolic breakdown is occurring under enzymatic control is provided by observing the rate and conjugation moieties used during breakdown. Bensulfuron is metabolized approximately 10 times faster in tolerant than susceptible species (Takeda et al., 1986). Sulfometuron is hydroxylated at faster rates in tolerant compared to susceptible species (Anderson and Swain, 1992). Metabolism of triflusulfuron and rimsulfuron occurs ~35 times faster in sugar beets and maize, respectively, compared with sensitive species (Koeppe et al., 2000; Wittenbach et al., 1994). Nicosulfuron metabolism also proceeds faster in tolerant maize compared with sensitive maize (Simpson et al., 1994). Some SU are conjugated with glutathione which indicates involvement of GSTs (see above). Metabolism of triflusulfuron in sugar beet is thought to be controlled by a GST, since the metabolites contain glutathione conjugates and appear at least 35 times faster in tolerant compared to sensitive plants (Wittenbach et al., 1994). Glutathione conjugation has also been observed in chlorimuron treated soybeans and peanuts (Ferguson, 1988; Brown and Neighbors, 1987). The position of glutathione conjugation on chlorimuron differs in each species, indicating differences in the GSTs. Flupyrsulfuron metabolism in wheat cultivars is thought to be regulated by GSTs, since the major metabolite contained a glutathione conjugate (Barefoot and Christensen, 1995).

Further supporting evidence of enzymatic control of SU breakdown is provided by induction of metabolism. Chlorimuron is not metabolized rapidly in maize, however metabolism can be enhanced in maize treated with the safener BAS 145 138 (Lamoureux and Rusness, 1992). Safened (BAS 145 138) maize exhibited an increase in chlorimuron metabolites hydroxylated at the 5 position of the pyrimidine ring (Lamoureux and Rusness, 1992). The hydroxyl group at the 5 position rendered the structure susceptible to conjugations with glucose, and glutathione conjugations at the 4 position of the pyrimidine ring (Lamoureux and Rusness, 1992).

The bridges in SU herbicides are susceptible to pH dependent cleavage and contraction, therefore this is associated with chemical breakdown (Roberts et al., 1998). Evidence of enzymatic bridge contraction was reported with flazasulfuron (Yoshii, 1993). Bridge cleavage was more rapid in tolerant plants compared with sensitive plants (Yoshii, 1993). Nicosulfuron, chlorsulfuron, and tribenuron will undergo bridge cleavage as well as hydroxylation and glucose conjugation in maize and wheat, respectively (Roberts et al., 1998). Rate of bridge cleavage of sulfosulfuron treated wheat was dependent on the application. POST applications of sulfosulfuron result in primarily unmetabolized herbicides (90%), while PRE application resulted in more (40%) metabolites with cleaved bridges (Nadeau and Kurtzweil, 1994). Cinosulfuron is metabolized in rice through bridge cleavage, aryl hydroxylation, and O-demethylation of the triazine moiety (Roberts et al., 1998). It is uncertain how much enzymatic activity contributes to the breakdown of cinosulfuron in rice.

The mechanism of breakdown of some SU are unlike any other. In rape ethametsulfuron is primarily degraded by dealkylation at the N and O substituents on the triazine ring (Roberts et al., 1998). The breakdown rate in tolerant rape is 2 to 15 times faster than in sensitive species, and therefore is suspected to be controlled enzymatically (Lichtner et al., 1995). In tolerant plants, thifensulfuron is metabolized to a herbicidal inactive metabolite through methyl ester hydrolysis (Roberts et al., 1998).

Different SU are transformed through different mechanisms in the same plant. Different plants will transform the same SU at different positions on the herbicide or by different mechanisms. The mechanisms of SU transformations are as diverse as the class of herbicides itself. Therefore predicting the transformation of SU in plants is difficult at best.

Xenobiotic Metabolism in tomato and pepper

There are few herbicides registered for use in tomato and pepper in the United States. However, studies on mechanisms of herbicide tolerance have been conducted in tomato and pepper. Tomato has been shown to metabolically transform metribuzin, napropamide, and diphenamid (Frear et al., 1983; Hodgson et al., 1973).

Some tomato cultivars are tolerant to metribuzin (Gawronski, 1983; Stephenson et al., 1976). Tolerance of tomato to metribuzin is influenced by growth stage and genetic line (Gawronski, 1983; Stephenson et al., 1976; Silva and Warren, 1976). Tomato requires 16 times more metribuzin to reduce fresh weight when sprayed with 7 true leaves as opposed to 1 (Silva and Warren, 1976). Stephenson et al. (1976) and Gawronski (1983) demonstrated differential tolerance with 41 tomato cultivars. Stephenson et al. (1976) further correlated the whole plant tolerance to differential metabolism between two cultivars. Frear et al. (1983) determined that metabolites of metribuzin treated tomato contained a glucose moiety for every ^{14}C molecule separated from the parent material. Frear et al. (1976) indicated that the metribuzin was metabolized from a UDP-glucose:metribuzin *N*-glucosyltransferase, based on enzyme assays that produced identical metabolites found in tomato leaves. If metabolism of metribuzin is controlled enzymatically, then the expression or activity of the enzyme is not static or present in all cultivars.

Tomato metabolized 95% of the supplied napropamide through conjugation with hexose (Murphy et al., 1973). Napropamide metabolism in tomato also appears to depend on tissue. Green callus cultures were more tolerant to napropamide than white cultures

(Zilkah, et al., 1978). Diphenamid is transformed in tomato to several metabolites (Hodgson et al., 1973). Diphenamid is initially transformed to *N*-hydroxymethyl-N-methyl-2,2-diphenylacetamide (MODA), and then thought to under go linkage with a glucoside moiety (Hodgson et al., 1973). Further conjugation with glucose has been observed and transformation into insoluble residues has been proposed (Hodgson et al., 1974).

Pepper is tolerant to clomazone while tomato is not. Clomazone metabolism was studied in tomato and pepper to determine its role in crop selectivity (Weston and Barrett, 1989). Clomazone metabolism was greater in the roots of pepper, while tomato metabolized more in the shoots (Weston and Barrett, 1989). The metabolites in both species co-chromatographed indicating similar transformations. However, these metabolites were not assayed for inhibition of the Shibata shift, so metabolism maybe the mechanism of selectivity. Pepper is not tolerant to metribuzin and does not metabolize this herbicide to appreciable quantities. Tolerance differences between tomato and pepper to metribuzin could be attributed to metabolism.

Pepper was similar to tomato in the transformation of diphenamid. In both cases the N on the benzene rings were altered by reductive processes and then further stabilized through addition of a glucose moiety (Hodgson and Hoffer, 1977). It appears tomato will convert more diphenamid into water soluble (glucose conjugated) metabolites in a 24hr period than pepper (28 vs 3 %) (Hodgson and Hoffer, 1977; Hodgson et al., 1973). Pepper is tolerant to diphenamid despite the slower transformation compared to tomato.

Herbicide/Insecticide Interactions

The transformation of herbicides in higher plants can be negatively affected by other pesticides. The organophosphate and carbamate insecticides have been primarily identified in reducing crop tolerance of multiple classes of herbicides in different plants (Holshouser et al., 1991; Morton et al., 1991; Matsunaka, 1968; Swanson and Swanson, 1968). Much of the insecticide/herbicide interaction studies have centered around terbufos and maize. Terbufos is a soil applied insecticide that is used in maize to control rootworms (*Diabrotica* spp.), and consequently the negative interactions between this insecticide and herbicides have a major impact on food production in the United States.

It is known that the absorption of organophosphates or carbamates will reduce the transformation of herbicides to non-phytotoxic metabolites in plants (Baerg et al., 1996; Holshouser et al., 1991; Matsunaka, 1968; Swanson and Swanson, 1968). The mechanism by which terbufos interferes with cytochrome P-450 metabolism of herbicides is under investigation with each herbicides. Terbufos has been shown to interfere with *in vitro* metabolism of herbicides, indicating the interference is occurring directly with the enzymes responsible (Baerg et al., 1996; Diehl et al., 1995). The similarity between the structures of terbufos, and its derivatives, with herbicides metabolized by CYPP450s has led to speculation that competition for the active site of the CYP450 enzyme occurs. Baerg et al. (1996) proposed the terbufos metabolites may act as suicide inhibitors to cytochrome P450s and the herbicide and insecticide must be metabolized by the same enzyme. In maize it is likely that some SU and terbufos are metabolized by the same cytochrome P450. Diehl et al. (1995) established nicosulfuron and terbufos have similar physiochemical properties.

Cytochrome P450 mediated herbicide metabolism is a common phenomenon that has been well documented in monocots (Lau and O'Keefe, 1996). However, negative interactions between herbicides and insecticides have been reported in dicots. Tolerance and subsequent yield was reduced when terbufos and metribuzin were applied to soybeans (Waldrop and Banks, 1983). Tomato tolerance to propanil was reduced in the presence of organophosphate insecticides.

There are few examples demonstrating dicot *in vitro* metabolism of herbicides. Monocot microsomal assays are conducted by inducing the herbicide detoxifying enzymes by pretreatment of tissue with either safner or ethanol. Herbicide detoxifying enzymes in dicots do not respond to the same induction treatments, and consequently microsomal fractions are more difficult to achieve from dicots. It is assumed that the same enzymes that detoxify herbicides in monocots are at work in dicots, since some of the metabolites are structurally similar.

In summary, plants tolerate herbicides (including the SU) through target site insensitivity, herbicide metabolism, and limited uptake and/or translocation (Lua and O'Keefe, 1996; Radosevich et al., 1997). Herbicide rate and environmental conditions play a minor role in herbicide tolerance as it relates to plant mortality. Physiological maturity of plants when a herbicide is applied and insecticides have a greater influence on tolerance and mortality. Generally speaking, most crop plants tolerate SU through rapid transformation to non-phytotoxic metabolites (Koeppe and Brown, 1995). Transformations of sulfonyl ureas can occur through many different pathways.

The need for selective herbicides for tomato and pepper production will increase with the loss of methyl-bromide in 2005. Little is known on how tomatoes or peppers tolerate herbicides in general, and SU herbicides in particular. The mechanism of tomato tolerance to SU needs to be determined. Past research and experience has shown knowing the mechanism of crop selectivity can aid in predicting negative environmental and insecticide interactions with herbicides. Furthermore, understanding the mechanism of crop selectivity is the base for future progress for overcoming herbicide sensitivity. It is obviously possible to overcome pepper sensitivity to SU by inserting genes conferring herbicide tolerance.

The objectives for this research focused on the characterization of tomato tolerance to rimsulfuron and halosulfuron, by comparison with the sensitive species pepper.

(1) Tolerant and sensitive cultivars of tomato and pepper will be identified for use throughout the study.

(2) Tomato varietal tolerance to rimsulfuron and halosulfuron and interactions with insecticides will be investigated.

(3) Tomato and pepper ALS sensitivity to rimsulfuron and halosulfuron will be characterized. Foliar and root uptake and translocation of these two SU will be compared in tomato and pepper.

(4) Rimsulfuron metabolism will be investigated in both species.

CHAPTER 3
TOMATO (*Lycopersicum esculentum* Mill.) CULTIVAR SENSITIVITY TO RIMSULFURON AND HALOSULFURON

Introduction

Differential sensitivity of crop cultivars and weed biotypes to sulfonylureas (SU) is well documented (Ackley et al., 1999; Mathiassen et al., 1999; Urban and Adamczewski, 1999; Augustin et al., 1998; Green, 1998; Solymosi and Nagy, 1998; Williams and Harvey, 1996; Bewick et al., 1995; Sterling and Jochem, 1995; Green and Ulrich, 1993; Newson and Shaw, 1992; Stall and Bewick, 1992). Bewick et al.(1995) specifically documented varietal sensitivity of tomatoes to rimsulfuron. Many of the tomato cultivars tested in 1995 are no longer commercially used. Halosulfuron is currently receiving a section 18 label for use in Florida for fresh market tomatoes. Therefore, fresh market cultivars that are currently in commercial use should be tested for tolerance to rimsulfuron and halosulfuron. Testing of cultivars currently used will provide a better prediction of the performance of these herbicides under wide spread commercial use.

Nine tomato cultivars ('FL 47', 'FL 91', 'Equinox', ' BHN 444', 'Mountain Spring', 'Carolina Gold', 'Celebrity', 'Captiva' and 'Cherry grande') were selected to screen for rimsulfuron and halosulfuron tolerance, based on breeding programs and popularity of use. Most of the cultivars were jointed and determinate, but they do vary in their season of maturity and vigor, which is influenced by the climate they are grown in. 'FL 47', 'FL 91', 'Captiva', and 'Equinox' are determinate and jointed cultivars (Hochmuth et al., 2000). In University of Florida trials, 'FL 47' and 'FL 91' produced moderate to high yields, predominately large fruit, with a low percentage of culls. 'Captiva' is a new cultivar similar to 'Sunbeam' in horticultural characteristics (moderate mid season yields on determinate plants), but with improved disease resistance. 'Mountain Spring' and 'Carolina Gold' are also jointed determinate cultivars, but are suited for use in the cooler climates of the Midwest and northeast United States (Syngenta Seeds Inc., 1998). 'Carolina Gold' is the more vigorous of the two cultivars (Syngenta Seeds Inc., 1998). 'BHN 444' is jointed and determinate, but is the only cultivar resistant to tomato spotted wilt commercially used in Florida (Hochmuth et al., 1999). 'Cherry Grande' produces “cherry” tomatoes on a medium sized determinate plant (Hochmuth et al., 2000). 'Celebrity' is a determinate tomato cultivar that is adapted to many climates.

The ability of 'Celebrity' to produce acceptable yields in many climates has made it one the most popular cultivars for home gardens. The commercial cultivars used in these trails were among the most commonly planted in Florida, during the 1999-2000 season (FASS, 2001). The objectives of this study were to (1) establish the relationship between tomato cultivar and halosulfuron tolerance, and (2) determine the extent of rimsulfuron sensitivity in current tomato cultivars.

Materials and Methods

Tolerance of these cultivars to halosulfuron and rimsulfuron was tested in the greenhouse in the fall of 1999 and 2001, respectively. Pots in all experiments measured 14 x 15 cm. Each pot was filled to an equal volume using an organic based commercial potting media (MetroMix 220®). Seeds of ' BHN 444', 'Captiva', 'Carolina Gold', 'Celebrity', 'Cherry grande', 'Equinox', 'FL 47', 'FL 91', and 'Mountain Spring' were obtained from commercial lot sources C9708, 1018, HT8154, 887480-2512, 1112, 1008, 12877, 14841-1, and IT 7123, respectively. Seeds of each cultivar were sown into trays with 1" cells, and later manually transplanted into the pots used for the experiment. Cultivars were transplanted to a depth equal to the existing soil line. One plant of each cultivar was transplanted into a pot and watered to field capacity. Transplanting of tomatoes used in the halosulfuron/cultivar study occurred on October 5 and 20, 1999. In the fall of 2001, the tomatoes used in the rimsulfuron/cultivar study were transplanted on September 1 and November 3. The experiment initiated on October 5, 1999 was replicated five times, while the experiment initiated on October 20, 1999 was replicated four times. Both studies conducted in the fall of 2001 were replicated five times. After transplanting, the plants were grown for 40 days. During this growing period, plants were irrigated every other day, by directly pouring water onto the soil until reaching field capacity. Nutrients were applied using a commercially formulated 20-20-20 fertilizer every three days mixed at a concentration of 236 ppm of N, until completion of the experiment. A total of 0.5g of nitrogen 0.22g of phosphorus, and 0.41g of potassium, were applied during each experiment.

Herbicide treatments were applied POST, one week after transplanting. Rimsulfuron rates were 0 and 0.0175 kg/ai ha. Halosulfuron rates were applied at of 0 and 0.045 kg/ai ha. A nonionic surfactant, X-77 ®, with an HLB of 13, was included in the spray solution at a concentration of 0.25% to optimize rimsulfuron and halosulfuron activity (Green and Green, 1993).

Plants were rated every 10 days after application until harvest. After 40 days the shoot matter was harvested, by cutting the shoots at the soil surface. Fresh weights were recorded immediately after harvest. Analysis of variance (ANOVA) was used to test for interactions of experimental timing and treatment effects. Duncans procedure was used to separate means of each cultivar in response to the herbicide treatment.

The tolerance of three cultivars 'BHN 444', 'Mountain Spring', and 'FL 47' to halosulfuron was evaluated by measuring yield in response to herbicide rate. This study was conducted during the spring of 2000 at the North Florida Research and education center (Quincy, Fl.). This study was designed as a split plot. The main plots were cultivar and the sub-plots were herbicide treatment. There were four replications for each treatment. Treatments were halosulfuron applied at 0 (as a control), 0.027kg/ha, and 0.045kg/ha. All treatments included a non-ionic surfactant at the concentration of 0.25% v/v. Tomato transplants were manually transplanted in April 1, 2000. Tomatoes from each cultivar were harvested twice on June 19th and 27th 2000. Harvested fruit was graded for defects, and then separated by size (diameter) into medium, large, and extra-large fruit. Once separated by size, the fruit was weighed and recorded. Defected fruits were weighed and recorded as cull fruit. All data were subjected to analysis of variance to detect significant treatment effects.

Results and Discussion

Halosulfuron and Tomato Cultivar trial

In the experiment conducted on October 5, 1999 not all five replicates survived the transplanting process, therefore the design was unbalanced. The fresh weight data from both experimental runs were transformed to a percent of control, by dividing the weight of plants treated with halosulfuron with the non-treated control for each replication of each cultivar. Then data from each halosulfuron cultivar study was pooled and, using the type III sum of squares, the significance of experimental run on treatment and cultivar was determined. Analysis of variance indicated the time the experiment was conducted did not significantly affected the treatment or the cultivar. The three-way interaction between experimental run, cultivar, and treatment was also not significant. Data from the two experimental runs was pooled and further analyzed. Halosulfuron, tomato cultivar, or the interaction between the two did not significantly influence tomato biomass accumulation. The fresh weight means from each cultivar were subjected to Duncans procedure, which did detect some differences in the tomato cultivars when analyzing the percent of control.

'FL 47', 'FL 91', and 'Captiva' accumulated the greatest amount of biomass during the experiment (Table 3-1). However, Duncans procedure did not reveal any significant differences between the means of cultivars treated with halosulfuron. When the transformed data (percent of control) were analyzed Duncan's procedure did indicate differences in the means of the cultivars treated with halosulfuron. Among the nine cultivars tested 'Celebrity' had the largest percentage of the control and was significantly better than all other cultivars except 'Captiva' and 'BHN 444' (Table 3-2). The cultivar that accumulated the lowest biomass compared with the control was 'Mountain Spring', and it was only 18% lower than the control (Table 3-2). This data indicates that the nine tomato cultivars tested exhibited some differences, but excellent tolerance to halosulfuron. Furthermore, cultivars that have been reported as being vigorous, such as 'Celebrity', did respond better to herbicide applications.

Fruits were harvested twice in the field experiment. In this experiment the total yields for 'FL 47', 'Mountain Spring', and 'BHN 444' were 41,752, 39,805, and 55,994 kg/ha^{-1}, respectively. The majority of the yields for all three cultivars were large and extra-large fruit. The average state yield of tomato for 1999-2000 growing season was 40,292 kg/ha^{-1}, therefore the yield in this experiment was acceptable.

The data from both harvests were analyzed separately and then combined and analyzed as a total yield. When data from the first harvest were analyzed by ANOVA, results indicated medium and large fruit were not significantly affected by the halosulfuron applications or cultivar. The extra-large fruit were significantly affected by the main plot tomato cultivar, but not the sub-plot halosulfuron treatment. Results from the second harvest were similar to the results from the first harvest. None of the fruit sizes were significantly affected by the halosulfuron treatments, but the large and extra-large fruit were significantly affected by tomato cultivar (main plot). When harvests were combined for the total yield, ANOVA revealed the main plot (cultivars) significantly affected the yield of extra-large sized fruit and the sub-plot (halosulfuron) did not affect any of the yield of other fruit sizes (Figure 3-3). Of all the fruit harvested in the cultivar 'BHN 444' 54% was extra-large fruit, compared to 45 and 49% in the other cultivars. Medium sized fruit was not significantly affected by cultivar, which is apparent in Figure 3-2. All three cultivars are similar at each point on the x axis. Yield of large sized fruit was significant at the 10% level, but not the 5% (Figure 3-1). There were some differences between the cultivars in the control plots, but not in the treated halosulfuron plots. The lack of significant effect of halosulfuron on yield of all three cultivars is represented in Figures 3-3.

Rimsulfuron/Tomato Cultivar trial

Fresh weight data from the experiments analyzing rimsulfuron influence on tomato cultivars were transformed to the percent of control as described above, due to the enate differences in the growth of cultivars (Table 3-2). Transformed data were pooled and then tested for appropriate interactions between experimental runs, cultivar, and treatment. ANOVA indicated the time the experiment was conducted did not significantly affect the treatment or cultivar. The three-way interaction between experimental run, cultivar, and treatment was not significant. Therefore data was pooled and ANOVA was used to determine the significance of herbicide treatment and cultivar on tomato growth.

The application of rimsulfuron at 0.0175 kg/ai ha did not significantly reduce the biomass of any of the tomato cultivars tested. Further analysis using Duncan's procedure on the transformed (percentage of the control) means, indicated all the cultivars used accumulated equivalent biomass.

'Celebrity' did not accumulate as much biomass in the rimsulfuron/tomato cultivar trial, while 'FL 47' accumulated more, compared with the previous halosulfuron/tomato cultivar trials. The average maximum and minimum temperatures for the rimsulfuron/cultivar experiment were 41 and 9.5^0C, respectively. This is similar to the average maximum and minimum temperatures recorded in the halosulfuron/cultivar experiment, which were 42.5 and 7^0C, respectively. Watering schedules were the same in all experiments (see above), therefore growth differences of the tomatoes in these experiments appear to be associated with factors other than environmental conditions.

One commercial tomato cultivar used in these trials was selected to be used in all other studies (herbicide/insecticide trials, ALS assays, uptake and translocation, and metabolism). Results from these trails were evaluated to select the most common cultivar (in terms of acreage), with the highest vigor, and lowest phytotoxicity after treatment with halosulfuron or rimsulfuron. Based on these criteria, 'FL 47' was selected as the tomato cultivar to be used in all other experiments. The pepper cultivar 'X3R Camelot' was selected to represent the species *Capsicum annuum*. This cultivar was selected based on commercial acreage planted. The tolerance of these cultivars to rimsulfuron and halosulfuron will be compared.

Table 3-1. Tomato Halosulfuron Cultivar Trial

Cultivar	Mean Percentage Weight of Control	Mean Weight (g)
'BHN 444'	101 ab	55 a
'Captiva'	105 ab	67 a
'Carolina Gold'	99 b	59 a
'Celebrity'	142 a	41 a
'Cherry grande'	90 b	50 a
'Equinox'	91 b	49 a
'FL 47'	94 b	71 a
'FL 91'	94 b	74 a
'Mountain Spring'	82 b	51 a

Different letters indicate statistically different means at the $\alpha=0.05$ level.

Table 3-2. Tomato Rimsulfuron Cultivar Trial Fall, 2001

Cultivar	Mean Percentage Weight of Control	Mean Weight (g)
'BHN 444'	95.29 a	111.6 a
'Captiva'	104.54 a	51.69 de
'Carolina Gold'	99.8 a	97.06 ba
'Celebrity'	99.5 a	64.94 dc
'Cherry grande'	102.8 a	43.08 e
'Equinox'	89.83 a	31.19 e
'FL 47'	112.52 a	84.56 bc
'FL 91'	99.71 a	31.72 e

Different letters indicate statistically different means at the $\alpha=0.05$ level.

CHAPTER 4

INTERACTIONS OF INSECTICIDE WITH SULFONYLUREA HERBICIDES IN TOMATO (*Lycopersicum esculentus* Mill.) AND PEPPER (*Capsicum annuum* L.) CULTIVARS

Introduction

Organophosphates and carbamates have been shown to interfere with the enzymes in plants responsible for metabolizing herbicides and thereby reducing tolerance to the herbicides (Holshouser et al., 1991; Morton et al., 1991; Matsunaka, 1968; Swanson and Swanson, 1968). Studies were conducted to examine potential interactions between the insecticides terbufos (organophosphate) and oxamyl (carbamate) with the sulfonylureas rimsulfuron and halosulfuron. The insecticide/herbicide interactions studies were initiated for two reasons. If negative interactions between the insecticides and herbicides are observed in the tolerant species tomato, then it indicates the mechanism by which tomato is tolerant to the sulfonylureas. Oxamyl is a carbamate insecticide that provides control of nematodes and is currently registered for use in fresh market tomatoes in Florida. With the banning of methyl bromide, vegetable production in Florida will need to rely on alternative control measures for soil borne pests. Since oxamyl is a carbamate that will most likely be used more frequently in the absence of methylbromide, its compatibility with the newer sulfonylureas (SU) should be tested on several commercial tomato cultivars.

The objectives of this study were (1) to establish a sensitive pepper cultivar to use in future studies. The next objective was (2) to establish any negative interactions observed when oxamyl or terbufos were applied with rimsulfuron or halosulfuron on tomato or pepper, and thereby providing evidence that metabolism is involved in the tolerance of tomatoes to the sulfonylureas herbicides. The last objective was (3) to determine if tomato tolerance is negatively impacted by the use of oxamyl, and rimsulfuron or halosulfuron on several commercial tomato cultivars. The study objectives were accomplished through a series of greenhouse and field trials.

Materials and Methods

Greenhouse Studies

Plants were grown in pots measuring 14 x 15 cm in all greenhouse experiments. In greenhouse experiments nutrients were provided using a commercially formulated 20-20-20 fertilizer. A total of 0.5g of nitrogen 0.22g of phosphorus, and 0.41g of potassium, were applied during each experiment. Irrigation was applied as needed to maintain the soil near field capacity. After transplanting each experiment was conducted for 40 days. Plants were rated every 10 days after application until harvest, when shoots were harvested at the soil surface and biomass was weighed and recorded.

Studies examining terbufos interactions with rimsulfuron and halosulfuron were conducted during the spring of 1999. In these studies, the cultivars 'FL 47' and 'X3R Camelot' were used for tomato and pepper, respectively. Plants were transplanted into the pots on the May 17, 1999 and watered to field capacity. Each pot was filled equally based on volume with sand. The sand was used to minimize the binding of terbufos to organic matter.

Treatments were rimsulfuron or halosulfuron applied POST. Rimsulfuron rates were 0, 0.0175, 0.035, 0.07, 0.14, and 0.28 kg/ai ha with and without terbufos. Halosulfuron rates were 0, 0.024, 0.032, 0.040, and 0.080 kg/ai ha. A non-ionic surfactant, with a HLB of 13, was included in all POST applications of rimsulfuron or halosulfuron at the rate of 0.25% to optimize activity (Green and Green, 1993). Terbufos was applied at a rate equivalent to1.1 kg/ha (0.5g/pot), in the bottom of the transplanting hole.

Studies examining the interaction between oxamyl and halosulfuron or rimsulfuron were tested in the greenhouse in the fall of 1999 and 2001, respectively. Each pot was filled to an equal volume using an organic based commercial potting media (MetroMix 220®). Seeds of ' BHN 444', 'Captiva', 'Carolina Gold', 'Celebrity', 'Cherry Grande', 'Equinox', 'FL 47', 'FL 91', and 'Mountain Spring' were obtained from commercial sources from lots C9708, 1018, HT8154, 887480-2512, 1112, 1008, 12877, 14841-1, and IT 7123, respectively. Seeds of each cultivar were sown into trays with 1" cells, and later transplanted manually into the pots used for the experiment. Cultivars were transplanted to a depth equal to the existing soil line. One plant of each cultivar was transplanted into a pot and watered to field capacity. Transplanting of tomatoes used in the halosulfuron and oxamyl study occurred on October 5 and 20, 1999. In the fall of 2001, the tomatoes used in the rimsulfuron and oxamyl studies were transplanted on

September 1 and November 3. The experiment initiated on October 5, 1999 was replicated five times, while the experiment initiated on October 20, 1999 was replicated four times. Both studies conducted in the fall of 2001 were replicated five times.

Oxamyl was applied at the rate of 0.56 and 1.1 kg/ai ha ten and three days before herbicide application. Herbicide treatments were applied POST, ten days after transplanting. Rimsulfuron rates were 0 and 0.0175 kg/ai ha. Halosulfuron rates were applied at the rate of 0 and 0.045 kg/ai ha. A nonionic surfactant, with an HLB of 13, was included in the spray solution at a concentration of 0.25% to optimize rimsulfuron and halosulfuron activity (Green and Green, 1993).

Analysis of variance was used to test for interactions of experimental timing and treatment effects. The GR_{50} is a measure of the herbicide concentration required to reduce the above ground biomass by 50%, and this was used to compare sensitivity between species for each herbicide tested. The GR_{50} was calculated, as described by Hall et al. (1998), where the means of shoot weights reduced more, less than, and 50% (of the control) are used to create a linear regression model. This regression model then predicts the herbicide concentration required to reduce the shoot biomass 50%. The effect of herbicide treatment within a species was described by using regression models. Colby's method was used to detect synergistic herbicidal effects from the herbicide and insecticide combinations.

Field Studies

The effect of oxamyl and halosulfuron on tomato yield was examined in three cultivars 'BHN 444', 'Mountain Spring', and 'Fl 47'. This study was conducted during the spring of 2000 at the North Florida Research and Education Center (Quincy, Fl.). This study was designed as a split plot. The main plots were cultivar and the sub-plots were herbicide and insecticide treatments. There were four replications for each treatment. Treatments were halosulfuron applied at 0 (as a control), 0.027kg/ha, and 0.054kg/ha, with and without 0.56 kg/ai ha of oxamyl. All treatments included a non-ionic surfactant at the concentration of 0.25% v/v.

Plants were grown on black polyethylene mulch, and supplemental nutrition was provided before mulch was laid. Nitrogen, phosphorus, and potassium were added to soil in a 13-4-13 formulation, at the rate of 1,680 kg/ha of product. Supplemental irrigation was provided throughout the experiment through drip tape.

Tomato transplants were manually transplanted in April 1, 2000. Tomatoes from each cultivar were harvest twice on June 19th and 27th 2000. Harvested fruit was graded for defects, and then separated by size (diameter) into medium, large, and extra-large fruit. Once separated by size, the fruit was weighed and recorded. Defected fruits were weighed and recorded as cull fruit. Vigor ratings were recorded every fifteen days on a 0 to 100 scale, where 0 = plant death and 100 = growth similar to that of the control plants. All vigor data were adjusted, using an arcsin transformation, before further analysis. All data were subjected to analysis of variance to detect significant treatment effects.

Regression models were used to characterize significant effects. Regression models used were determined empirically based on the data in each trial.

Results and Discussion

Data from trials examining the response of the tomato and pepper cultivar 'FL 47' and 'X3R Camelot', respectively, to halosulfuron and rimsulfuron treatment were subjected to analysis of variance (ANOVA). ANOVA was first used to determine if the time the trials were conducted significantly affected the data. The time each trial was conducted did not significantly influence tomato or pepper response to halosulfuron or rimsulfuron. Data were pooled and analyzed with ANOVA, to determine if the species, treatment, or the interaction between the species and treatment were significantly affecting the shoot dry weight. Species and its interaction with halosulfuron and rimsulfuron significantly affected the shoot weight. Therefore data were separated by species. Pepper shoot weight was significantly affected by halosulfuron and rimsulfuron, while tomato was not. Data from both species were regressed against herbicide rates (Figure 4-1 to 4-3).

A logistic model best described the relationship between shoot weight of tomato and pepper to increasing rimsulfuron rates. The equations for the models describing tomato and pepper response to rimsulfuron were $Y=1.01+(\log)-0.69x$ and $Y=0.003+(\log)-1.14x$, respectively, with $r^2$of 0.99. It was apparent that tomato was more tolerant than pepper. The GR_{50} for the pepper cultivar used in these trials was reached at 0.01kg ai ha^{-1} of rimsulfuron, compared with a GR_{50} of 0.28 for the tomato cultivar used. This represents a 28-fold increase in tolerance of 'FL 47' over 'X3R Camelot'. The results of this trial demonstrate the differences observed in these cultivars is similar to the differences previously reported between these species (Stall, 1999). Based on the model, when rimsulfuron is applied alone to tomato and the rate is increased there is a concurrent decrease in the shoot weight. Examination of the data points regressed and the error associated with each mean reveals that there is little reduction in shoot weight between the rates. The tomato shoot weight was not significantly reduced from the control until the rate of 0.28 kg ai ha^{-1}, which is eight times the labeled use rate in Florida. Furthermore, the difference in tolerance (28-fold) between these species is similar to the tolerance level reported in some weeds that have become resistant to herbicides (Buker et al., 2002). Therefore, these cultivars were determined to be useful for studies investigating tolerance differences between a tolerant tomato and a sensitive pepper species to rimsulfuron.

A sigmoidal model best described the relationship between pepper and halosulfuron rate (Figure 4-2). The equation for the model used was $Y=56+460*\exp(((x-0.012)/0.011)^2)$ which had a r^2 of 0.85. The GR_{50} for 'X3R Camelot' was 0.023 kg ai ha^{-1}. Below this rate there is little herbicidal activity on any plant species. Therefore it is apparent that the pepper cultivar 'X3R Camelot' is not tolerant.

Tomato exhibited excellent tolerance to halosulfuron. No halosulfuron rate reduced the shoot weight compared with the control (Figure 4-3). ANOVA results confirm that halosulfuron had no significant effect on tomato shoot weight ($p>0.05$). There was no reduction in the shoot weight of the tomato cultivar 'FL 47', so a GR_{50} could not be obtained. The highest rate of halosulfuron used was 0.08 kg ai ha^{-1}, so 'FL 47' must be at least 3.47 times more tolerant to halosulfuron than 'X3R Camelot'.

The data from the trials using halosulfuron and rimsulfuron indicates, at the whole plant level, the tomato and pepper cultivars selected are more sensitive to rimsulfuron than halosulfuron. Pepper is more sensitive to both of these herbicides than tomato, and tomatoes tolerate these herbicides at rates that cause death in other plant species. Therefore, these two cultivars will be used as model species for further investigation into tolerance differences between these species.

Terbufos/Herbicide Interactions

Data from studies investigating tolerance mechanisms of tomato and pepper to rimsulfuron were analyzed to determine if plants treated with terbufos and rimsulfuron affected tolerance and therefore shoot weight. Results from ANOVA of the rimsulfuron terbufos study indicated that there were significant interactions between the time each experiment was conducted, species, and the treatments. However data could be pooled by species across time. The sub-sets of data were further analyzed by ANOVA to determine if the herbicide, insecticide, and the combinations significantly affected shoot weight. Results from ANOVA indicated that rimsulfuron and the interaction between rimsulfuron and terbufos were significant in tomato, but not pepper. Therefore the data from tomato were separated by insecticide and regressed against rimsulfuron rate (Figure 4-4). The response of tomato shoot weights to rimsulfuron and rimsulfuron plus terbufos were empirically determined to best fit a logistic models.

The models describing the response of tomato shoot weight to rimsulfuron and rimsulfuron plus terbufos were Y=0.597 + (log)-0.771x (r^2 0.95) and Y= 0.698 + (log)-0.252x (r^2 0.92), respectively (Figure 4-4). The reduction in shoot weight predicted by the model decreases as the rimsulfuron rate increases. This is similar to what was observed in previous studies (Figure 4-1).

When plants were treated with terbufos prior to rimsulfuron applications there is a significant reduction in the shoot weight compared to shoot weights of tomato treated with rimsulfuron alone. Treating tomatoes with terbufos prior to rimsulfuron application negates the rate effect in a negative manner. Shoot weight of tomato treated with terbufos and 0.018kg/ai ha was reduced 72% compared with the control, and increasing the rate to 0.28kg ai ha^{-1} only reduced the weight an additional 12% (84% reduction). The effect of terbufos in reducing tomato tolerance to rimsulfuron is evident at the lower rates. At the labeled rates of 0.018 and 0.035 kg/ai ha, terbufos can reduce tomato shoot weights by 69 and 42%, respectively (Figure 4-4).

The reduction of herbicide tolerance by terbufos has been reported with other SU in field and sweet corn. Terbufos reduces SU tolerance in maize, by by reducing herbicide metabolism (Robinson et al., 1996; Siminszky et al., 1995; Simpson et al., 1994; Morton et al., 1991). Terbufos interferes with cytochrome P-450s, which are thought to be involved in the metabolism of SU in maize and wheat (Frear et al., 1991; Fonne-Pfister et al., 1990). Waldrop and Banks (1983) demonstrated a negative interaction of metribuzin and terbufos in soybeans. However, they did not indicate or report the cause of this negative interaction. The data gathered from terbufos interaction with rimsulfuron is the first reported in tomato, therefore the cause of the negative interaction observed in our trials can be only assumed to be caused by reduced metabolism. If the negative interaction observed here is caused by reduced metabolism, then rimsulfuron metabolism in tomato may also be mediated by a cytochrome P-450.

There was not a significant interaction between rimsulfuron and rimsulfuron plus terbufos treatments in pepper, so the data was pooled and regressed against rimsulfuron rate. Pepper response to rimsulfuron was also empirically determined to best fit a logistic model (Figure 4-5). The data symbols of rimsulfuron and rimsulfuron plus terbufos were graphed independently to highlight the similarity between the treatments. The response of pepper to foliar applications of rimsulfuron was poor. Pepper was reduced 90% with the lowest rate (0.018kg/ai ha). The addition of terbufos did not decrease pepper tolerance, but terbufos applied by itself did reduce pepper shoot weight 66%. The data from the studies on tomato and pepper response to rimsulfuron and rimsulfuron plus terbufos, collectively indicate that metabolism may play a role in the whole plant tolerance differences.

Data from studies investigating the tolerance mechanisms of tomato and pepper to halosulfuron were analyzed to determine if the addition of terbufos affected the tolerance and resulting shoot weight. Results from ANOVA of the halosulfuron/terbufos study indicated that there were significant interactions between species. Data from like species could be pooled by across time, and was for further analysis. Further analysis of tomato data, using ANOVA, indicated that halosulfuron significantly affected the shoot dry weight, but terbufos and the interaction between terbufos and halosulfuron did not. When halosulfuron rate increased there was initially no response in shoot weight, until 0.036 kg ai ha^{-1}, at which point the dry weight actually increased. Halosulfuron did significantly affect the shoot weight of tomato, but in a positive manner.

Halosulfuron did significantly reduce the shoot weight of pepper, but the addition of terbufos did not significantly increase pepper sensitivity. As with the previous study with halosulfuron, there was little decrease in shoot weight after 0.027kg ai ha^{-1}. This is similar to what was observed with pepper treated with rimsulfuron and terbufos.

No negative interaction from halosulfuron and terbufos was observed with tomato. The lack of a negative interaction indicates the tolerance mechanism of tomato with halosulfuron may be different from the mechanism exhibited with rimsulfuron. However, some assumptions must be made to predict tomato is exhibiting a different mechanism of tolerance to halosulfuron and rimsulfuron based on this data. The first assumptions must be that the form and amounts of terbufos absorbed in each study were the same. The inhibitive effects of terbufos can be altered by its form and availability. The form of terbufos, like other pesticides, is influenced by temperature. At higher temperatures more terbufos exists as sulfonamide derivatives, which is more inhibitive to some species (Chapman and Harris, 1980; Laveglia and Dahm, 1975). Terbufos is also susceptible to binding to organic matter (specifically cation exchange sites) in the soil (Chapman and Harris, 1980; Laveglia and Dahm, 1975). In our studies using rimsulfuron and halosulfuron the average air temperatures were similar 48 -19^0C and 43 to 23^0C maximum and minimum, respectively (Appendix A). The assumption was made that the soil temperature would be similar as well, and therefore the form of terbufos entering the tomato was the same when rimsulfuron and halosulfuron was tested. The media used in the pots was sand, and had little organic matter, but the organic matter was not quantified. Similar amounts of terbufos were assumed to be available in both studies.

Greenhouse Oxamyl/Herbicide Interaction

Data from studies investigating tolerance mechanisms of tomato and pepper to rimsulfuron were analyzed to determine if plants treated with oxamyl and rimsulfuron affected tolerance and therefore shoot weight. Results from ANOVA indicated that the time each experiment was conducted did not influence the results, therefore data from both experiments were pooled and analyzed for interactions between herbicide, insecticide, and cultivar. There was no significant effect from the combinations of rimsulfuron or oxamyl at the rates tested on any of the ten cultivars (Table 4-2.).

Data from halosulfuron studies were analyzed to determine if plants treated with oxamyl and halosulfuron affected tolerance and therefore shoot weight. Results from ANOVA indicated that the time the experiments were conducted did not influence the results, therefore data from both experiments were pooled and analyzed for interactions between herbicide, insecticide, and cultivar. There was only a significant negative effect from the combination of rimsulfuron and oxamyl on one of the ten cultivars tested (Table 4-3). Shoot weight of 'Carolina Gold' was reduced when oxamyl and halosulfuron were applied to the plant. It is difficult to determine if this reduction is shoot weight was caused by pesticide interaction, because the significant affect occurred at the lower rate of oxamyl but not at the higher.

Results from studies investigating the tolerance of tomato to halosulfuron and rimsulfuron in conjunction with oxamyl indicate that these herbicide insecticide combinations are safe to use. The safety of oxamyl and halosulfuron and rimsulfuron combinations also indicate, if there is a mediated breakdown of these herbicides in tomato, then inhibitors of this breakdown are limited to organophosphate insecticides.

Field Oxamyl and Herbicide Interactions

Cull data from field studies investigating interactions between oxamyl and halosulfuron were analyzed by ANOVA to determine if the distribution of the culls were affected by treatments, cultivar, or interactions of the two. Cultivar significantly affect the cull weight during the first harvest, but the treatment or cultivar and treatment interaction did not significantly affect culls. Culls accounted for only 15 to 18% among any of the cultivars during the first harvest. Furthermore, the culls harvested during the second and total yield data (combinations of first and second harvests) were not significantly affected by any variable. The majority of the yields of all of the harvest were distributed between the large and extra-large fruit (between 71 to 82% for all harvest and cultivars). This yield data is similar to yield data reported by others for these cultivars (Hochmuth et al., 2000; Maynard, 2000; Novartis Seeds Inc., 1998). The total yields for 'FL 47', 'Mountain Spring', and 'BHN 444' were 41,752, 39,805, and 55,994 kg/ha^{-1}, respectively. These yields are comparable to the average yield of tomato in Florida during the 1999-2000 growing season (40,292 kg/ha^{-1}). The yield and fruit size distribution of each cultivar indicates the growth and yield of tomato were comparable to conditions in commercial tomato production in Florida.

Data were further analyzed with ANOVA to determine if the applications of halosulfuron, oxamyl and the combinations had a negative impact on tomato growth or yield. Vigor of tomato plants were not affected by halosulfuron, oxamyl or the combinations of the two at any rate tested. Vigor was affected by the cultivar, 'BHN 444' tended to have a higher vigor than the other cultivars. At the last harvest, the number of plants in each plot were counted to adjust for losses from Tomato Spotted Wilt Virus. Applications of oxamyl did significantly increase the number of plants present at the end of the season.

Halosulfuron and oxamyl combinations had negligible impacts on tomato plant vigor and yields. According to ANOVA the combination of halosulfuron and oxamyl did not impact yield of extra-large, large, or medium fruit during any of the harvests or the total yield data. The same results were also found when halosulfuron treatment was analyzed. ANOVA could not detect any significant affect on the medium, large, or extra-large fruit from any of the harvests or the total yield data.

Trends of increased fruit number and weight were observed when the data from oxamyl treatments were analyzed. At the first harvest tomato plants treated with oxamyl had a slightly higher harvest of large fruit compared to plants not treated, 7.6 versus 6.6 kg/plot, respectively. However, this trend (p=0.0604) was not significant at the p=0.05 level.

Data from the second harvest revealed similar trends. When plants were treated with oxamyl the yield of medium fruit and number of medium fruit was significantly increased 1.2 kg/plot and 14.8 fruit/plot, respectively. The yield of large fruit from plants treated with oxamyl also increased, however not significantly (p=0.109). Furthermore, the interaction between cultivar and oxamyl for number of large fruit was almost significant (p=0.0616). The yield of large fruit from 'Mountain Spring' increased from 5.6 kg/plot to 8.6 kg/plot when treated with oxamyl. Comparatively small changes from the other two cultivars in large fruit yield was observed (<0.6 kg/plot). Analysis of the second harvest data did reveal a significant effect in the large fruit number. Cultivar and oxamyl significantly interacted. The number of large fruits from 'Mountain Spring' increased from 33 fruit/plot to 53 fruit/plot when treated with oxamyl. The affect of oxamyl on the second harvest was still apparent on the total yield. Oxamyl significantly increased total medium and large fruit yields 1.35 and 2 kg/plot, respectively. The change in large fruit yield from the other two cultivars at the second harvest were nominal (<2 fruit/plot).

The increase in the medium and large fruit yield could be attributed to a lack of stress. Morales-Payan (1999) found the majority of tomato yield losses, from purple and yellow nutsedge competition, occurred in the medium sized fruit. Methyl bromide was not applied to the field where this study was conducted, so the oxamyl could be reducing a negative effect from a pests (nematode or insect).

Cultivar significantly affected various yield parameters throughout this study. During the first harvest cultivar was found to significantly impact medium fruit number, extra-large fruit yield and number. Data from the second harvest revealed cultivar affected large and extra-large fruit yield and number.

From these experiments a cultivar of pepper ('X3R Camelot') and tomato ('FL 47') that were highly sensitive and tolerant, respectively to rimsulfuron and halosulfuron was identified and quantified. With respect to tomato, some insight was provided into the mechanism of tolerance of rimsulfuron and halosulfuron, through the terbufos herbicide interaction studies. No prior examples could be found in the literature reporting negative interactions from organophosphates and SU herbicide tolerance in broadleaf crops. This study provides an example of organophosphate interfering with sulfonylurea tolerance in a broadleaf. This system suggests a novel pathway for SU metabolism is the mechanism of differential tolerance.

The studies with oxamyl and rimsulfuron or halosulfuron has established that these products are safe to use. No negative interactions would be expected form the use of these products at the rates tested on many of the cultivars currently used in Florida tomato production.

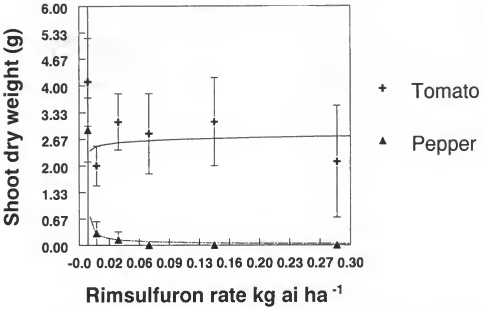


Figure 4-1.Tomato and pepper shoot weight regressed against rimsulfuron, using a logistic model.

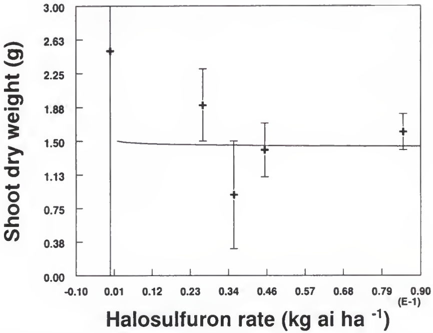


Figure 4-2. Pepper shoot weight regressed against halosulfuron, using a sigmoidal model.

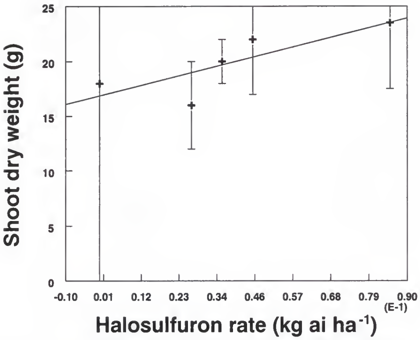


Figure 4-3. Tomato shoot weight regressed against halosulfuron, using a linear model.

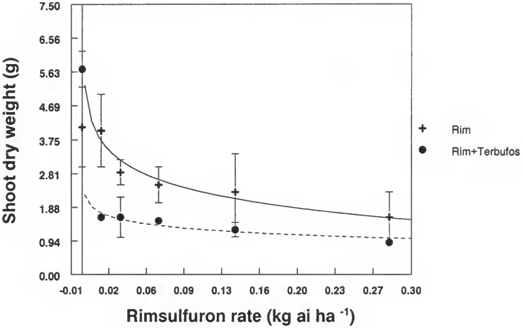


Figure 4-4. Tomato shoot weight regressed against rimsulfuron and rimsulfuron plus terbufos.

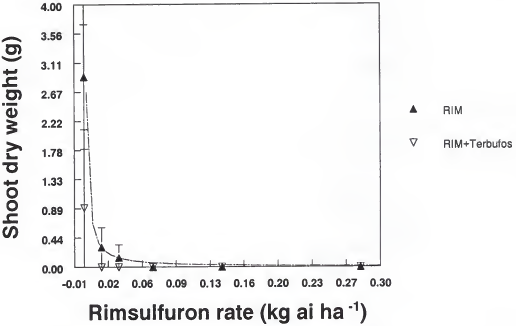


Figure 4-5. Pepper shoot weight regressed against rimsulfuron and rimsulfuron plus terbufos, using a logistic model.

Table 4-1. Comparison of fresh weight (g) of tomato shoots treated with combinations of halosulfuron and oxamyl. ns=no significant difference between treatments and ** indicates significant affect (α=0.05).

	Oxamyl	Halosulfuron		
		0.0	.045 kg/ai ha	
'FL 47'	0.56 kg ai ha^{-1}	57	55	ns
	1.1 kg ai ha^{-1}	66	58	ns
'Mountain Spring'	0.56 kg ai ha^{-1}	63	50	ns
	1.1 kg ai ha^{-1}	58	71	ns
'Carolina Gold'	0.56 kg ai ha^{-1}	71	45	**
	1.1 kg ai ha^{-1}	59	63	ns
'BHN 444'	0.56 kg ai ha^{-1}	62	72	ns
	1.1 kg ai ha^{-1}	55	75	ns
'Cherry Grande'	0.56 kg ai ha^{-1}	39	61	**
	1.1 kg ai ha^{-1}	49	59	ns
'FL 91'	0.56 kg ai ha^{-1}	54	52	ns
	1.1 kg ai ha^{-1}	52	43	ns
'Equinox'	0.56 kg ai ha^{-1}	59	40	ns
	1.1 kg ai ha^{-1}	60	59	ns
'Captiva'	0.56 kg ai ha^{-1}	62	73	ns
	1.1 kg ai ha^{-1}	50	54	ns
'Celebrity'	0.56 kg ai ha^{-1}	62	72	ns
	1.1 kg ai ha^{-1}	45	65	ns

Table 4-2. Comparison of fresh weight (g) of tomato shoots treated with combinations of rimsulfuron and oxamyl. The symbol ns = no significant difference between treatments and ** indicates significant difference (α=0.05).

	Oxamyl	Rimsulfuron		
		0.0	.045 kg/ai ha	
'FL 47'	0.56 kg ai ha^{-1}	76	74	ns
	1.1 kg ai ha^{-1}	86	80	ns
'Cherry Grande'	0.56 kg ai ha^{-1}	43	39	ns
	1.1 kg ai ha^{-1}	52	39	ns
'Fl 91'	0.56 kg ai ha^{-1}	32	36	ns
	1.1 kg ai ha^{-1}	34	31	ns
'Equinox'	0.56 kg ai ha^{-1}	48	32	ns
	1.1 kg ai ha^{-1}	29	36	ns
'Captiva'	0.56 kg ai ha^{-1}	35	67	ns
	1.1 kg ai ha^{-1}	42	42	ns
'Celebrity'	0.56 kg ai ha^{-1}	58	70	ns
	1.1 kg ai ha^{-1}	64	43	ns
'Carolina Gold'	0.56 kg ai ha^{-1}	103	85	ns
	1.1 kg ai ha^{-1}	97	81	ns
'BHN 444'	0.56 kg ai ha^{-1}	60	92	ns
	1.1 kg ai ha^{-1}	106	97	ns

CHAPTER 5

ACTIVITY AND INHIBITION OF TOMATO (*Lycopersicum esculentum* Mill.)AND PEPPER (*Capsicum annuum* L.) ACETOLACTATE SYNTHASE BY HALOSULFURON AND RIMSULFURON

Introduction

When considering all of the mechanisms of tolerance for each plant/herbicide combination, an insensitive target site is the second most frequent mechanism responsible for a plant tolerating a given herbicide (Devine et al., 1993). Ten different plants have been reported that are resistant to ALS or ACCase inhibiting herbicides, because of target site mutations. In the case of one triazine resistant weed, the insensitivity at the target site was a mutation that rendered the resistant plant less "fit" and this weed is only found in ecosystems where the herbicide is frequently used (Holt et al., 1993; Radosevich et al., 1997). The evolved plants that are resistant to ALS and ACCase herbicides are not less "fit." The similar "fitness" between sensitive and insensitive species indicates the mutated and sulfonylurea insensitive enzymes function within the needs of the plant allowing normal growth and reproduction (Prado et al, 2000; Baumgartner et al., 1999; Manley et al., 1999; Boutsalis and Powles, 1995; Kwon and Penner, 1995; O'Donovan et al., 1995; Marshall et al., 1992; Christopher et al., 1992; Saari et al., 1992; Devine et al., 1991). The tolerance difference between tomato and pepper reported by Stall (1999) could be a result of altered target site sensitivity. The sensitivity of tomato and pepper ALS was investigated to (1) compare enzyme sensitivity of these species with sensitive and insensitive ALS from species reported in the literature and (2) to correlate the target site (ALS) sensitivity of tomato or pepper with whole plant tolerance.

Materials and Methods

Enzyme Extraction Procedures

The ALS extraction procedures were adopted from Singh et al. (1988). Only immature and recently matured leaves from plants six to eight weeks old were removed and used in the ALS extractions. All glassware was pre-chilled and extraction procedures were conducted under chilled conditions to protect enzymes. Other protective measures included in the extraction buffer were protease inhibiting cocktail, DTT, PVPP, and XAD-4 (Appendix B). The extraction buffer contained EDTA to remove cations that could interfere with ALS activity (Tse and Schloss, 1993; Davies, 1964; Satyanarayana and Radhakrishnan, 1962). FAD, TPP, leucine, pyruvate, and valine were added to the extraction buffer to stabilize the ALS (Appendix B). Davies (1964) indicated that ALS had the best activity with Mg^{++} as the cation, therefore $MgCl_2$ was added to the extraction medium. The ALS was concentrated by adding polyethylene glycol, and centrifuging the extract. The final pellet was re-dissolved in a potassium-phosphate based buffer. This buffer contained DTT, FAD, and $MgCl_2$ to protect the ALS. The pellet was then run through a column containing sephadex G-25, to remove small molecules. The column was loaded and the sample was eluted using a potassium-phosphate based buffer

(100mM) containing 100mM of FAD. Elution and collection of the protein were monitored using a Bio-Rad ECONO UV monitor. Collected protein was stored in a -80^{0}C freezer until use. Concentrations, the sources of the reagents, and the volume of the buffer used in the extraction process can be found in Appendix B. The protease inhibition cocktail was made of 16 different inhibitors, which are listed in Appendix B. The pump and monitor settings are detailed in Appendix B.

ALS Assay

ALS is an enzyme that catalyzes the first step in the synthesis of valine, leucine, or isoleucine. When pyruvate is the only substrate added in this assay, the synthesis of leucine and valine is favored. Acetolactate is an intermediate in the synthesis of these amino acids. Singh et al. (1988) used a method described by Westerfield (1945) to convert acetolactate, produced from ALS, into acetoin. When acetolactate is incubated at 60^{0}C with creatine and α-napthol it will chemically convert to acetoin. The acetoin can be quantified in a spectrophotometer, and therefore used to quantify the ALS activity.

The optimum pH, protein content, temperature, and time for ALS assays were determined for pepper and tomato. Using potassium-phosphate buffers the activity of ALS was assayed over the pH ranges of 6.0 to 8.0. Optimum ALS activity of both tomato and pepper was observed at pH 7.5.

Singh et al. (1988) reported increased ALS activity with increasing temperatures. The optimum temperature for tomato and pepper ALS activity was determined by assaying activity of both species between 25 and 45^0 C. The assay temperature used for both species was 40^0 C, which is similar to what others have reported (Singh et al., 1988) (Appendix C).

With herbicides added to the assay, the activity decreases and reduces the sensitivity of the assay. The assay time was set to be long enough to provide a high level of activity, while still being in the linear portion of the activity curve. The assay time was empirically determined to be ran for 90 minutes, which is similar to what has been used in other species (Butler and Siehl, 1992; Rattray et al., 1994; Singh et al., 1988; Relton et al., 1986; Davies, 1964; Satyanarayana and Radhakrishnan, 1963) (Appendix D).

For consistency and ease the Bradford method was used to determine the protein content. Bradford dye and BSA were used to create a standard curve to estimate the protein content in our samples. The effect of protein content on ALS activity of both species was examined, to determine the optimum level of protein to add to assays to obtain maximum activity. To be in the linear portion of the activity curve and obtain maximum activity all assays contained less than 400μg/assay (Engel, 1996) (Appendix E).

All assays were conducted with a potassium-phosphate based buffer, FAD, TPP, $MgCl_2$, and sodium pyruvate. The co-factors FAD, TPP, and $MgCl_2$ were included in the assay mixture at saturating levels (Appendix F). New stocks of halosulfuron, rimsulfuron, creatine, α-napthol, FAD, TPP, and sodium pyruvate were made the day each assay was conducted. Procedures used in preparing these stocks are detailed in Appendix F.

Activity of tomato and pepper ALS was characterized and compared by assaying enzymes from each species under varying substrate and inhibitor concentrations. The substrate (sodium pyruvate) concentrations used in the assay mixtures were 20, 16, 10, 8, and 4mM. Technical grade halosulfuron was mixed at the concentrations of 0, $0.5x10^{-10}M$ and $0.5x10^{-9}M$ for tomato protein extracts, while assays of pepper protein extracts received 0, to $8x10^{-7}M$. Rimsulfuron was mixed at the concentrations of 0 to $1x10^{-7}M$ and added to pepper protein extracts, while tomato protein assays received 0 to $1x10^{-8}M$. Assays were conducted in 15x125 mm glass test tubes. Test tubes were placed in ice water-bath, while assay mix, then substrate and herbicide treatment, and finally protein extracts were added. Each treatment was duplicated three times and a heat-killed assay was used as a control. Heat-killed assays were made by adding the enzyme to a test tube pre-heated and maintained at 100^0 C, and heating for fifteen minutes. Then the rest of the mixture was added to the test tube.

Enzyme activity was assayed at 40^0C, by placing the test tubes in a water-bath for 90 minutes. All assays were stopped by adding 6N H_2SO_4 and heating in a 60^0C water-bath for fifteen minutes. After the reaction was stopped, creatine (0.5% wt/v) and α-napthol (5% wt./v) were added to each assay mixture and incubated for an additional 15 minutes at 60^0 C. Test tubes were removed to stand at room temperature for 30 minutes to develop maximum color (Westerfield, 1945). After 30 minutes, at room temperature, assay tubes were centrifuged for 1 minute at 14,000 rpm, to remove debris that introduced variation in samples (Tipton, 1995).

Samples were analyzed using a Beckman DU® 520 spectrophotometer with the wave length set at 525 nm. Specific activity of each sample was calculated to make treatment comparisons between and within species. Specific activity was calculated by subtracting the background absorbance value from the treatment absorbance to give the corrected absorbance. The corrected absorbance was adjusted for dilution by multiplying the corrected absorbance by ten. Then the corrected value was multiplied by 1000 to adjusted the value to mg. The corrected value was then divided by the total μg of protein used in the assay to adjust for protein concentration, and then multiplied by 0.1416 to obtain a μmolar value for the specific activity.

Data were then plotted to determine if the enzyme activity fit Michaelis-Menton kinetics. Data that fit the pattern of Michaelis-Menton kinetics were plotted in double reciprocal plots and used in Leonora® (Cornish-Bowden, 1995) to estimate the inhibition patterns, apparent Km, Vmax, and K*i*s.

Results and Discussion

ALS Activity

Data collected from experiments observing halosulfuron and rimsulfuron inhibition of extractable ALS activity of tomato and pepper, was plotted against sodium pyruvate concentration. Data from each experiment followed Michaelis-Menton kinetics ($V=Vmax[S]/Km+[S]$). The enzyme activity from tomato and pepper were slightly different, with respect to the substrate sodium pyruvate. Based on the Km_{app} and $Vmax_{app}$, tomato ALS activity was consistent between extractions used for rimsulfuron and halosulfuron inhibition experiments. Using the same criteria, the double reciprocal plots and Leonora® indicates pepper ALS $Vmax_{app}$ differed between extractions for rimsulfuron and halosulfuron experiments (Table 5-1). The differences between the pepper ALS activity in the rimsulfuron and halosulfuron studies were not large enough to disregard the data.

Comparing the velocity at which acetolactate was formed by the activities of pepper and tomato ALS, the conversion of sodium pyruvate to acetolactate in the presence of pepper ALS is faster. Specific velocity of extractable ALS from pepper was higher than tomato. Further evidence of differences between tomato and pepper ALS activity is provided when comparing the Km and Vmax of the species. The differences in activity between these species were noted, and experiments examining their ALS activity in the presence of rimsulfuron and halosulfuron were conducted.

Data gathered from these experiments did not indicate the presence of isozymes affected by varying pH in either species. Singh et al.(1988) reported that the isozymes found in Black Mexican sweet corn had different pH optima. The isozymes, AHAS (or ALS) I and AHAS II, had optimal activity in a range of pH 6-7 and distinctly at 7.0, respectively (Singh et al., 1988). Davies (1964) likewise reported pH optima of 7.0 and 8-8.5 for two ALS isozymes from pea. In both tomato and pepper, there was only one distinct peak in activity corresponding to the pH 7.5 (Figure 5-1). Two isozymes could optimally function at the same pH. Therefore, this data does not discount the possibility that isozymes of ALS are present in tomato or pepper. The presence of isozymes doesn’t appear to be important, since ALS in both species are susceptible to inhibition from rimsulfuron and halosulfuron.

ALS Inhibition

Double reciprocal plots and least squares method of analysis showed mixed inhibition of pepper and tomato ALS with rimsulfuron (Table 5-2) (Figure 5-2; 5-3; 5-4; 5-5). SUs typically inhibit ALS in higher plants through what is described as noncompetitive inhibition. Mixed inhibition is similar to noncompetitive inhibition. With both inhibition patterns, the inhibitors can bind to both the enzyme and the enzyme/substrate complex. In both the noncompetitive and mixed inhibition, the inhibitor is acting upon a site other than the enzyme/substrate reaction site (Zubay, 1998).

Higher concentrations of rimsulfuron were required to inhibit ALS of pepper compared with tomato. A concentration of $1\text{x}10^{-9}$M slightly increased pepper ALS activity, while a similar concentration inhibited the activity of tomato ALS (Figure 5-2 and 5-3). A concentration of $1\text{x}10^{-8}$M rimsulfuron inhibited tomato ALS activity 90%, similar reductions (90%) in pepper activity occurred only after a ten fold increase in rimsulfuron concentration.

The differences in rimsulfuron concentration required to inhibit ALS activity of both species was evident in the Ki_{app} as well. With rimsulfuron as an inhibitor, Leonora® predicted a higher Ki_{app} for pepper than tomato ALS. The higher Ki_{app} means the ALS of pepper is more tolerant to rimsulfuron than tomato (Table 5-2). Based on the Ki_{app}, the difference in enzyme sensitivity between the species to rimsulfuron is small. Saari et al. (1989) used R/S ratios for dry weight and ALS activity to determine the resistance mechanism of resistant and susceptible *Kochia scoparia* populations to metsulfuron-methyl. The R/S ratio for dry weight and ALS activity when sulfometuron-methyl was applied was 20 and 28, respectively. Saari et al. (1989) concluded the resistance of *Kochia scoparia* to metsulfuron-methyl was due to an insensitive ALS. Boustsalis and Powles (1995) compared an SU resistant *Sonchus oleraceus* L. biotype with a susceptible biotype. They reported findings similar to Saari et al. (1989) for sulfometuron inhibition of ALS (R/S 19). Using the same I_{50} R/S ratios for the ALS activity of pepper and tomato in the presence of rimsulfuron, pepper would be 10 times more resistant. At the whole plant level pepper was 28 times more sensitive to rimsulfuron than tomato (see Chapter 4).

As with the rimsulfuron data, tomato ALS appears to be more sensitive than pepper to the inhibitor halosulfuron. At the concentration of 0.5×10^{-9}M, halosulfuron reduced tomato ALS activity 67%, while pepper was reduced 52%. Based on the Ki_{app} estimates from Leonora®, tomato ALS was more sensitive to the inhibitor halosulfuron than pepper (Table 5-3). At the whole plant level pepper was at least 3.5 times more sensitive to halosulfuron than tomato (see Chapter 4).

The data presented strongly suggests that ALS insensitivity has little contribution to the tolerance differences between tomato and pepper to rimsulfuron or halosulfuron at the whole plant level. Whole plant tolerance of *K. scoparia*, *S. oleraceus*, and *Lactuca serriola* to SUs occurred when their ALS was 19 to120-fold more insensitive than susceptible biotypes (Eberlein et al, 1997; Boustsalis and Powles, 1995; Saari et al. 1989). The largest difference in the target enzyme sensitivity observed between the tomato and pepper was 10-fold, and the pepper was still sensitive at the whole plant level.

When comparing concentrations of inhibitors used in this study with studies of SU resistant *K. scoparia*, *S. oleraceus*, and *L. serriola*, then the sensitivity of ALS from both species to rimsulfuron and halosulfuron is more evident. First, different SUs (chlorsulfuron, metsulfuron-methyl, and sulfometuron-methyl) and higher concentrations were used with *K. scoparia*, *S. oleraceus*, and *L. serriola*, than the concentrations used with tomato and pepper. The concentrations of rimsulfuron and halosulfuron used in studies with tomato and pepper were between 0.05-100nM, which completely inhibited ALS activity in some cases (Figure 5-2 to 5-5). Eberlein et al. (1997) reported using 6,820nM of chlorsulfuron to inhibit ALS activity of SU resistant *L. serriola* 50%.

Concentrations that inhibit the activity of ALS are important to establish when relating ALS sensitivity to the mechanism of whole plant tolerance. No data establishing the concentration of any SU at the cellular level could be found. However, Joop and Amrhein (1986) reported 65,000nM of glyphosate was translocated into the stroma of spinach leaves from an assimilate exporting leaf. Glyphosate is applied at higher rates than halosulfuron or rimsulfuron, 16 to 35 times higher, respectively. If glyphosate, rimsulfuron, and halosulfuron exhibit equivalent uptake and translocation, then 4062 and 1857nM of halosulfuron and rimsulfuron, respectively could be expected in stroma. These concentrations are well beyond the levels of rimsulfuron or halosulfuron used to inhibit tomato or pepper ALS.

There were small differences between tomato and pepper in ALS sensitivity toward rimsulfuron and halosulfuron. The levels that inhibited the ALS of both species were much lower than what was reported in other resistant weeds (Eberlein et al., 1997;

Boustsalis and Powles, 1995; Saari et al. 1989). Neither species possesses an extractable insensitive form of ALS that renders the species tolerant to rimsulfuron or halosulfuron at the whole plant level.

Halosulfuron is a stronger inhibitor of extractable ALS of both tomato and pepper. With tomato ALS, Leonora® estimated the Ki_{app} of halosulfuron was 1.97 and 7.84 compared to 20.89 and 0.72 with rimsulfuron. Large differences were also observed when pepper ALS was used. Leonora® estimated the Ki_{app} of halosulfuron was 21 and 3.3 compared to 43.4 and 4.4 with rimsulfuron. However, less rimsulfuron is applied per hectare than halosulfuron to achieve a biological effect. The sensitivity differences between these species may reflect differences in metabolism, uptake or translocation.

Table 5-1. Estimations of Km_{app} (mM of pyruvate) and $Vmax_{app}$ (µmole/h/mg protein)of tomato and pepper, using double reciprocal plots and Leonora®. Each value represents the mean and values in the parenthesis represents the standard error ± , n=4.

	Double reciprocal plot		Leonora®	
	Km_{app}	$Vmax_{app}$	Km_{app}	$Vmax_{app}$
Tomato Experiment1	4.76 ± (1.47)	0.77 ± (0.13)	6.87 ± (1.75)	0.87 ± (0.09)
Tomato Experiment2	4.0 ± (0.76)	0.72 ± (0.19)	4.66 ± (0.82)	0.75 ± (0.04)
Pepper Experiment1	3.33 ± (0.16)	1.14 ± (0.53)	3.19 ± (0.33)	2.15 ± (0.06)
Pepper Experiment2	2.9 ± (0.87)	2.46 ± (0.76)	2.64 ± (0.40)	2.35 ± (0.10)

Table 5-2. Estimated Ki_{app} of rimsulfuron from Leonora®. Each value represents the mean and values in parentheses represents the standard error ±, n=4.

Estimated Ki_{app} of rimsulfuron		
	pepper	tomato
K*iu*	43.41nmoles ± (17.5)	20.89nmoles ± (7.65)
K*ic*	4.42nmoles ± (1.0)	0.72nmoles ± (0.27)

Table 5-3. Estimated Ki_{app} of halosulfuron using Leonora®. Each value represents the mean and values in parentheses represents the standard error ±, n=4.

Estimated Ki_{app} of halosulfuron		
	pepper	tomato
K*iu*	21.4nmoles ± (5.60)	1.97nmoles ± (0.53)
K*ic*	3.31nmoles ± (0.65)	7.84nnoles ± (10.44)

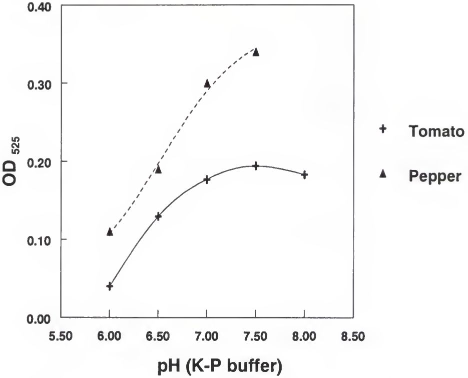


Figure 5-1. Tomato (+) and pepper (▲) ALS activity over varying pH ranges.

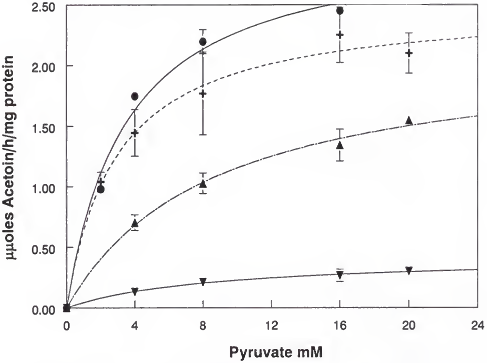


Figure 5-2. Pepper ALS inhibition by rimsulfuron. Rimsulfuron was used at 0 (+), 1x10^{-9}M (●), 1x10^{-8}M (▲), and 1x10^{-7}M (▼).

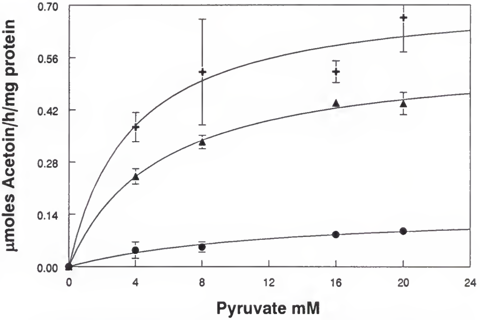


Figure 5-3. Tomato ALS inhibition by rimsulfuron. Rimsulfuron was used at 0 (+), 0.5x10^{-8}M (●), and 1x10^{-8}M (▲),

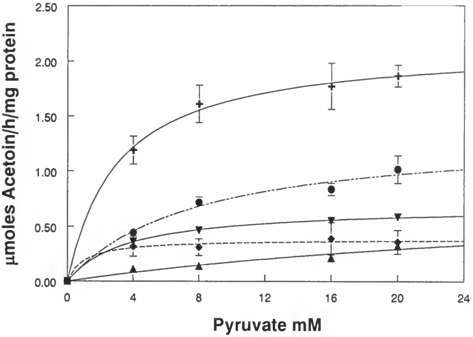


Figure 5-4. Pepper ALS inhibition by halosulfuron. Halosulfuron was used at 0 (+), 0.5x10^{-10}M (●), 0.8x10^{-9}M (◆), and 0.5x10^{-8}M (▼).

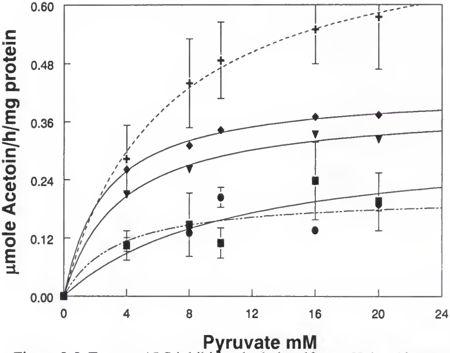


Figure 5-5. Tomato ALS inhibition by halosulfuron. Halosulfuron was used at 0 (+), $0.5\text{x}10^{-11}$M (◆), $0.5\text{x}10^{-10}$M (▼), $0.5\text{x}10^{-9}$ (■), and $0.5\text{x}10^{-8}$M (●).

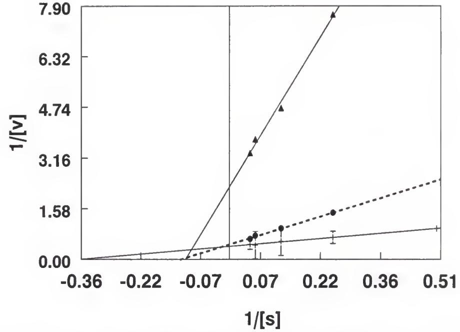


Figure 5-6. Double reciprocal plot of pepper ALS inhibition by rimsulfuron. Rimsulfuron was used at 0 (✚), 1x10^{-8}M (●), and 1x10^{-7}M (▲).

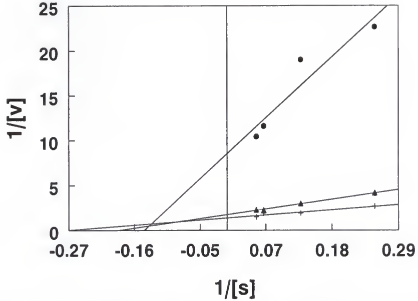


Figure 5-7. Double reciprocal plot of tomato ALS inhibition by rimsulfuron. Rimsulfuron was used at 0 (✚), 0.5×10^{-8}M (▲), and 1×10^{-8}M (●).

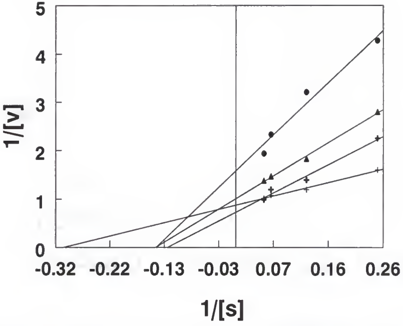


Figure 5-8. Double reciprocal plot of pepper ALS inhibition by halosulfuron. Halosulfuron was used at 0 (+), 1x10^{-8}M (✚), 5x10^{-8} (●), and 8x10^{-7}M (▲).

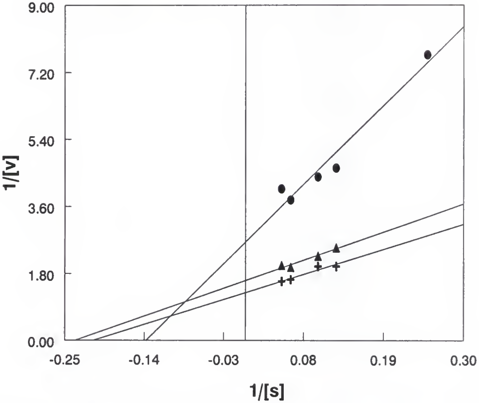


Figure 5-9. Double reciprocal plot of tomato ALS inhibition by halosulfuron. Halosulfuron was used at 0 (+), 1x10^{-8}M (▲), and 5x10^{-9} (●).

CHAPTER 6

UPTAKE AND TRANSLOCATION OF RIMSULFURON AND HALOSULFURON IN TOMATO (*Lycopersicum esculentum* Mill.) AND PEPPER (*Capsicum annum* L.)

Introduction

Limitations of herbicide uptake and translocation have been reported to aid in or actually be the primary mechanism of plant tolerance to some herbicides. Contributions of this mechanism to whole plant tolerance have also been reported in the newer SU herbicides. Decreased translocation of chlorsulfuron, metsulfuron, and triasulfuron was implied as one of the mechanism of resistance in two varieties of *Cirsium arvense* (Solymosi and Nagy, 1998). Soybean tolerance to thifensulfuron can be affected by translocation (Lycan and Hart, 1999). Soybean and two *Ipomoea* species are reported to tolerate chlorimuron by reduced uptake (Moseley et al., 1993). Rice tolerance to bensulfuron is aided, by reduced translocation when safeners are applied (Kwon and Pyon, 1993).

There are many factors that control the limited uptake and/or translocation of each combination of plant and herbicide. The two biotypes of *C. arvense* tolerant to chlorsulfuron, metsulfuron, and triasulfuron were reported to have thicker wax layers and fewer stomata on the leaf surface (Solymosi and Nagy, 1998). The morphological differences in the sensitive and resistant biotypes of *C. arvense* appear to control the tolerance of the biotypes to these herbicides. Some reductions in translocation can occur from chemical antagonism of the leaf surface. There are examples of antagonism of bentazon with other herbicides (Bellinder et al., 1997). Thifensulfuron translocation in soybeans was only reduced when applied in conjunction with bentazon. Applications of the combined herbicides do not decrease the rate of thifensulfuron metabolism, and the reduced translocation appears to be a secondary tolerance mechanism (Lycan and Hart, 1999).

The contribution of rimsulfuron and halosulfuron uptake and/or translocation to tomato tolerance or pepper sensitivity has not been reported. Furthermore, any possible mechanisms (morphological barriers versus physiological barriers) of differential herbicide uptake and translocation in tomato and pepper have not been reported in the scientific literature. Experiments were initiated with the objectives of examining stomatal densities between the two species. Rate of herbicide uptake when applied to the foliage and roots, and translocation of the herbicide overtime were determined.

Materials and Methods

Stomatal Density

Stomatal density between tomato and pepper was compared by using greenhouse and field grown plants. Comparisons between species were made by randomly selecting some recently matured leaves from five plants of each species. Each leaf from the plant species represented one replication. Leaves from each plant were cut at the petiole and brought into the laboratory to wash with deionized water. Acetate® (a proprietary mixture of butyl, ethyl, and glyceryl triacetate) was applied to the adaxial surface, allowed to dry, then the epidermis was peeled off using tweezers. Acetate peels were then placed on a microscope slide and observed using a Nikon eclipse E200® light microscope set at 40x power. All stomata present in the lens field being used (0.0062cm^{-2}) were counted and recorded.

Uptake and Translocation

Uptake and translocation of halosulfuron and rimsulfuron were monitored in tomato and pepper by using ^{14}C labeled herbicides in conjunction with non ^{14}C labeled herbicide. Both rimsulfuron and halosulfuron were labeled with ^{14}C on the pyridine and pyrazole rings, respectively. Three mg of ^{14}C labeled halosulfuron was provided by Nissan Chemical Industries, Ltd. 45, Miyukigaoka, Isukuba-shi, Ibaraki 305-0841, Japan, from lot# E-871202. The herbicide was 98.1% pure, with a specific activity of 2.12MBq/mg. Three mg of ^{14}C labeled rimsulfuron was provided by New England Nuclear Sciences, from lot# 3110-241A. The herbicide was 95.7% pure with a specific activity of 1.68MBq/mg.

Qualitative translocation was examined by pressing and drying plants at 50°C, and then exposing to X-ray sheets (Kodak X-OMAT AR® Rochester, NY 14650) for 40 days. X-Ray sheets were developed with a Kodak X-OMAT clinic 1 processor (Eastman Kodak Company, Rochester, NY). Radioactivity in uptake and translocation experiments was quantified by first sectioning the dried pressed plants and oxidizing the samples in a R.J. Harvey OX500 oxidizer (R.J. Harvey, Inc. Hillsdale, NJ). Actual radioactivity was quantified using a Beckman 6000 liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA) fitted with an internal standard. Procedures used in oxidizing the samples and calibrating the liquid scintillation counter with the R.J. Harvey cocktail fluid are listed in Appendix G.

Root Uptake and Translocation.

Uptake and translocation were monitored by applying the ^{14}C labeled herbicide with non-labeled herbicide simultaneously to the buffered solutions in which the plants were growing. Herbicide was allowed to absorb into the plants and translocate for 0, 24, 48, and 72 hours after application of herbicide (treatment). At the end of the treatment period the plants were removed from the buffered solutions containing the herbicide. Herbicide uptake was quantified by washing the roots at the end of the treatment period for 5 minutes with 10 mL of deionized water. Root wash was trapped in an Erlenmeyer flask, and 1 mL was removed and used to quantify remaining herbicide from the theoretical applied. Herbicide not detected in the root wash was assumed to be absorbed by the plant. After washing herbicide off the roots, the plants were placed on wax paper for drying and pressing. To prepare samples for oxidizing, plants were sectioned into roots, lower shoots and upper meristematic shoots.

Initially, plants were grown in 125 mL flasks wrapped in aluminum foil. The plants were maintained at a constant height (and light was prevented from entering the flask) by wrapping the stem of plants in cotton. Solution was 0.5 x Hoaglands buffered to pH 6.9 with a potassium-phosphate buffer. Solutions in each flask were aerated using a pump. Flasks and nutrient solutions were sterilized before use. Plants were transferred into flasks with solution and allowed to grow for one week prior to herbicide treatment. Plants used in the experiments with rimsulfuron were 27 to 34 days old. Plants used in experiments with halosulfuron were 35 to 40 days old. Experiments were conducted under artificial lighting. The average daily temperatures and light intensity are listed in Appendix G.

Technical grade rimsulfuron [>95% (not labeled with ^{14}C)] was dissolved in a 0.5mM potassium-phosphate buffer. The rimsulfuron labeled with ^{14}C was removed from the freezer allowed to come to room temperature and then 0.0787MBq was removed. Non-labeled (325nmoles) and ^{14}C labeled (7nmoles) rimsulfuron was added simultaneously to each flask.

Technical grade halosulfuron [>98% (not labeled with ^{14}C)] was dissolved in a 0.5mM potassium-phosphate buffer. The halosulfuron labeled with ^{14}C was removed from the freezer allowed to come to room temperature and then 0.133 or 0.178MBq was removed in the first and second experiments, respectively. Non-labeled (300nmoles) and ^{14}C labeled (7 or 9nmoles) halosulfuron was added simultaneously to each flask.

Foliar Uptake and Translocation.

Uptake and translocation was monitored by applying the ^{14}C labeled herbicide to leaf surfaces. The non-labeled herbicide mixtures were applied to the plants prior to the application of ^{14}C labeled herbicide, which ensured that the uptake of the ^{14}C labeled herbicide was monitored with a surfactant. Herbicided labeled with ^{14}C was applied to the adaxial surface of the most recently matured undamaged leaf. The herbicides were allowed to absorb and translocate for 0, 24, 48, and 72 hours after treatment [HAT(application)]. The area where ^{14}C labeled herbicide was applied was marked to aid in herbicide removal and uptake monitoring at the end of each treatment period. At the end of each treatment period the ^{14}C labeled herbicide was removed by washing the treatment area with 1 mL of deionized water five times. Leaf wash was trapped in a plastic vial, and 1 mL was used to quantify remaining herbicide from the theoretical applied. Herbicide not present in the leaf wash was assumed to be absorbed into the leaf tissue. After washing herbicide off the treated leaf, the leaf was cut at the petiole. Finally, plants were removed from the pot, to wash media off, and all plant tissue was placed on wax paper for drying and pressing. To prepare samples for oxidizing, plants were sectioned into treated leaf, shoots above treated leaf (meristem), shoots below treated leaf, and roots.

Plants were grown in plastic pots, measuring 11 x 8 cm. In the halosulfuron experiments, a sand media was used, while an organic based media, without perilite, was used in the experiments using rimsulfuron. Plants used in the experiments with halosulfuron and rimsulfuron were fertilized weekly with a 20-8.7-16 commercial fertilizer. Experiments were conducted under natural lighting in the same greenhouse. Air temperatures and light intensity of photosynthetically active radiation for the experiments are listed in Appendix G. The plants used in the first experiment with rimsulfuron were 20 days old and 25 days old in the second experiment. The plants used in the first experiment with halosulfuron were 30 days old and 21 days old in the second experiment.

Commercially formulated (25G) rimsulfuron (not labeled with ^{14}C) was mixed at a concentration of 0.0499 g/100 mL. A non-ionic surfactant, with a HLB of 13, was included in the rimsulfuron mixture at the concentration 0.25% v/v. The rimsulfuron labeled with ^{14}C was removed from the freezer allowed to come to room temperature and then 0.0084 MBq was removed per plant being treated. The stock solution was evaporated to dryness, and then redissolved in enough distilled H_2O to apply 2.5 μL per plant.

Technical grade halosulfuron [98%(not labeled with ^{14}C)] was mixed at a concentration of 0.3654 g in 1892 mL of deionized water (equivalent to 0.045kg/ha). A surfactant (X-77®) was included at 0.25% v/v. The halosulfuron labeled with ^{14}C was removed from the freezer, allowed to come to room temperature, and then 0.0067 MBq/treated plant was removed (11 μL). The stock solution was redissolved in 260 μL of distilled H_2O, which equates to 13 μL per plant.

Data from each experiment was pooled and analyzed with ANOVA ($p=0.05$) for interactions by experimental run. When there were no significant interactions between experiments, the data was combined. Where experimental runs significantly affected data, they were separated. Combined and separated data were further analyzed for significant treatment affects and interactions of species with treatment. Where species significantly affected treatment, data was separated by species. Significant treatment affects were analyzed through regression analysis.

Results and Discussion

Stomatal Density

The stomata density from greenhouse grown tomatoes were 20.6 ± 4.54 (0.0062 cm^{-2}). The density of greenhouse grown peppers were 17.6 ± 3.85 (0.0062 cm^{-2}). Statistical analysis indicated there were no differences in stomatal density of greenhouse grown tomato and pepper (Table 6-1). The same conclusion was drawn from the data from field grown tomato and pepper. Stomatal densities of field grown tomatoes were 16.2 ± 3.27 (0.0062cm^{-2}), while pepper were 17.8 ± 9.25 (0.0062cm^{-2}). There are examples of stomatal density and wax layer influencing whole plant sensitivity to SU (Solymosi and Nagy, 1998). From this example, uptake of the SU through stomata is implied. If halosulfuron or rimsulfuron were absorbed through the stomata, then the data presented here indicates differences in stomatal density would contribute little to the uptake and/or translocation process, since the density and size of each stomata are similar between each species (Table 6-1).

The difference between greenhouse and field grown tomatoes and peppers were investigated. No statistical differences were detected between plants grown in the two environments. Average number of stomata per lens field for greenhouse and field grown tomato was 20 and 16, respectively, while pepper was 17 for both environments. Data from these experiments indicate that foliar uptake and translocation experiments conducted with tomato and pepper plants grown under greenhouse conditions will have similar stomatal frequency. The thickness of the wax layer of greenhouse and field grown tomato and pepper plants was not investigated. Therefore not all morphological characteristics of the leaf that affects foliar uptake of herbicides were accounted for between tomato and pepper. The stomatal density and leaf weight between field and greenhouse tomato and pepper was similar under Florida conditions. Therefore, experiments investigating the contribution of uptake and/or translocation of halosulfuron and rimsulfuron in tomato and pepper were conducted under greenhouse conditions.

Root Uptake and Translocation

Halosulfuron. Halosulfuron root uptake data was analyzed with ANOVA as described in the material and methods section. The time the experiment was conducted affected the treatment. Data from each experiment was separated and further analyzed.

In both experiments treatment time and species significantly affected the uptake of halosulfuron through the roots. The interaction of species with treatment time did not significantly affect the root uptake of halosulfuron. Uptake data from each experiment was then regressed against treatment time.

In the first experiment, halosulfuron absorption in both species was linear with respect to time, so data was fit to a linear models (Figure 6-1). The maximum observed uptake of halosulfuron in tomato and pepper was 45 and 30% of the supplied herbicide, 72 HAT, respectively (Figure 6-1).

In the second experiment, halosulfuron absorption was best described by a linear and polynomial model in tomato and pepper, respectively (Figure 6-2). The observed uptake between the two species was similar up to 48 HAT (Figure 6-2). In pepper, the rate of uptake is predicted to decrease beyond 24 HAT, but as the incubation time passed beyond 48 HAT observed uptake rapidly increased in tomato. Maximum observed halosulfuron uptake by tomato and pepper was 24 and 11%.

Tomato and pepper absorbed similar amounts of halosulfuron in the first experiment. Furthermore, the rate of absorption over the first 24 HAT was similar (1.44 vs. 1.28 nmoles/hr in tomato and pepper, respectively). Differences in tomato and pepper absorption were observed in the second experiment, with tomato absorbing more herbicide than the pepper at 72 HAT. However, the rate of uptake during the first 24 HAT was similar between species, and also similar to the first experiment. Tomato and pepper absorbed 1.72 and 1.41 nmoles/hr, respectively, in the second experiment. This data indicates the increased tomato tolerance over pepper to preemergent applications of halosulfuron is not due to limited uptake.

The translocation of halosulfuron absorbed into tomato and pepper was investigated to determine if the herbicide moves similarly in these plants. Herbicides can be sequestered once taken up and never reach the target site (Shaner et al., 1998). Furthermore, the ALS content in meristematic regions have been reported to be higher than in other areas of the plant, so differential translocation between the species could alter the tolerance (Singh and Shaner, 1995).

Analysis of translocation data with ANOVA indicated the time each experiment was conducted significantly affected the translocation of the herbicide. This was expected since uptake was affected by the time the experiment was conducted. Data was also analyzed by the absolute halosulfuron found in each section. ANOVA of the absolute radioactivity indicated each section was not significantly different than the halosulfuron/mg of dry weight (dwt.) of plant, therefore this data was not shown.

In the first experiment, translocation was significantly affected by the treatment, species, and the section of the plant matter. Species did not significantly influence the effect of treatment or section on halosulfuron translocation.

In the first experiment the content of halosulfuron/mg dwt. of plant in the roots increased in a linear and exponential pattern in tomato and pepper, respectively (Figure 6-3). Halosulfuron content in tomato was significantly higher 48 HAT, but the maximum amount of halosulfuron is similar between the species. Furthermore, the maximum content of halosulfuron was found at the same treatment duration (72 HAT) in both species. In the first 24 HAT, tomato translocated 0.41 pmoles/mg dwt./hr compared with 0.46 pmoles/mg dwt./hr in pepper. The overall pattern of translocation may be different between the species, but the initial rate and final amount of herbicide translocated in the roots is similar.

The translocation of halosulfuron/mg dwt. in the middle shoots of the species occurred in a linear pattern (Figure 6-4). As with the data from the root section, maximal content of halosulfuron in the middle ⅓ of the plant was 72 HAT in both species (Figure 6-4). Maximum halosulfuron translocation into the upper ⅓ of tomato also occurred at 72 HAT, but was significantly higher in tomato than pepper (Figure 6-5).

It appears that tomato will translocate more radioactive material (herbicide and metabolites) than pepper. Furthermore, the rate of halosulfuron translocation is faster in tomato than pepper 24 HAT root absorption. In the middle sections of plants, tomato translocated 0.405 pmoles/mg dwt. of tissue/hr, which was about double the translocation rate in pepper (0.185 pmoles/mg dwt./hr). In the upper shoots of the plants, tomato translocated 0.21 pmoles/mg dwt./hr, while pepper translocated 0.14 pmoles/mg dwt./hr.

The majority of halosulfuron is found in the roots of the species at 72 HAT, but halosulfuron was transported to the upper ⅔ of tomato plants at a faster rate than in pepper. Overall, the data from the first experiment indicates herbicide sequestration does not contribute to the increased tolerance of tomato over pepper to preemergence halosulfuron applications.

In the second experiment, the treatment, species, and section significantly influenced the halosulfuron movement in plants. However, only the section significantly interacted with the treatment. Data was separated by species and section, then regressed against time.

In the second experiment, halosulfuron/mg dwt. of roots increased in a polynomial pattern in tomato and pepper (Figure 6-6). Halosulfuron levels increased up to 72 HAT in the roots. The variation at each time period suggests there are no differences between the species, but the rate of translocation into the roots in the first 24 HAT was not similar between the species. Tomato translocated 2.2 pmoles/mg dwt./hr while pepper translocated 1.46 pmoles/mg dwt./hr.

In the middle ⅓ of plants, levels of halosulfuron/mg dwt. of tissue increased in a linear pattern with respect to time (Figure 6-7). Halosulfuron movement into the middle ⅓ of the plants differed between the species 72HAT (Figure 6-7). Movement in pepper was more gradual over time, while there was a major increase in halosulfuron movement into the middle ⅓ of tomato between 48 and 72 HAT. This increase between 48 and 72 HAT at least indicates a higher metabolic activity in tomato. The initial translocation (0 to 24 HAT) was similar. Tomato translocated 0.18 pmoles/mg dwt./hr compared with 0.133 pmoles/mg dwt./hr in pepper. The initial rate and final amount of halosulfuron translocation into the middle ⅓ of plants was similar in corresponding species during the first and second experiment.

A positive linear relationship between movement of halosulfuron/mg dwt. of plant into the upper ⅓ of the plant and treatment duration was found in tomato and pepper (Figure 6-8). Translocation into the upper ⅓ was similar to the middle ⅓ of the plant. In the upper ⅓, significantly more halosulfuron was discovered in tomato than pepper 72 HAT, but the initial rate of translocation was higher in tomato than pepper (0.367 vs. 0.103 pmole/mg dwt./hr). The level of halosulfuron detected in the upper ⅓ of tomato is approximately 12% of the total amount detected in the plant compared to 5% in pepper. More meristematic cells are found in the upper ⅓ of plants than the middle ⅓. If all the radioactive material in tomato was untransformed halosulfuron, then translocation patterns exhibited in tomato would not favor tolerance.

Similar patterns of halosulfuron translocation, between the species was observed in the first experiment. Halosulfuron translocation to the upper ⅔ 72 HAT was not similar between the species in the second experiment, even though growth conditions during both experiments were similar.

When halosulfuron translocation was analyzed by the absolute halosulfuron (Bq) found in each section, there were different interactions compared with the data from halosulfuron/mg dwt. of plant. Absolute radioactivity in the roots of tomato and pepper increased over time, but the rate and area of halosulfuron accumulation differs between tomato and pepper. The halosulfuron levels in tomato doubled between 24 and 48 HAT in both shoot regions, compared to a gradual increase in pepper. Among the pepper shoots, the levels of halosulfuron were the highest in the upper shoots throughout all time periods. The skewed halosulfuron distribution in pepper shoots suggests transport occurred primarily in the xylem after root absorption, and was not moved via phloem to lower sections after absorption.

Autoradiographs of tomato and pepper were compared to access qualitative differences between the species. The darker areas in a autoradiograph represent where the radioactivity (halosulfuron) is located. When comparing tomato and pepper 72 HAT (Picture 6-1) the majority of the herbicide is contained in the roots of both species. In the shoots there seems to be an overall shading of the leaves, which indicates the herbicide has moved out of the vascular elements and into the leaf tissue of both species. Compared with pepper, tomato roots seem to be lighter and the shoots are darker, which indicates greater translocation into the shoots. However, the overall pattern of translocation is similar between the species. These findings coincide with the quantitative data from the liquid scintillation counter, where halosulfuron levels (radioactivity) were the highest 72 HAT.

Quantitative and qualitative data from the experiments suggests halosulfuron seclusion from the target site does not contribute to increased tolerance of tomatoes over pepper. Both experiments demonstrate halosulfuron uptake is no greater in pepper than tomato over 72 HAT. Once the halosulfuron is absorbed into the tissue there was little difference between the species in the amount moved, destination, or dispersion within sections. Where there was a difference in halosulfuron content in the sections, tomato accumulated more than pepper. The translocation data supports the notion that herbicide sequestration is not the primary mechanism for tolerance of tomato to preemergence halosulfuron applications.

Rimsulfuron. Statistical analysis of rimsulfuron root uptake data indicted the time the experiment was conducted did not affect the treatment, the response of each species to the treatment, or the interaction between species and treatment. The species did significantly affect the treatment, so data was separated by species and pooled over time. Further analysis of pooled data showed treatment affected uptake, so data was regressed against treatment.

Polynomial models were used to describe rimsulfuron uptake for tomato and pepper (Figure 6-9). As incubation time passed beyond 24 HAT the rate of rimsulfuron uptake in tomato rapidly increased six fold (5 to 30%) by 72 HAT. The uptake of rimsulfuron by pepper increased until 24 HAT, after this time the uptake was minimal up to 72 HAT. Maximum uptake of rimsulfuron in pepper was 13% 72 HAT (Figure 6-9). Uptake in pepper was greater than tomato in the first 24 HAT. Tomato and pepper absorbed 0.69 and 1.17 nmoles/hr, respectively. Absorption of rimsulfuron in pepper is flat beyond 24 HAT, which could be a herbicide induced response. Similar to observed halosulfuron uptake in the first experiment, rimsulfuron uptake was not linear over time in either species. Indicating passive diffusion is not the driving force behind rimsulfuron absorption.

Rimsulfuron translocation in tomato and pepper was investigated to determine its influence on whole plant tolerance. Initial analysis of translocation data indicated that the time each experiment was conducted did not affect the translocation of the herbicide in any way. Data from the experiments were pooled, and further analysis indicated the treatment significantly affected the rimsulfuron/mg dwt. of plant (Bq/mg dwt.).

Movement of rimsulfuron into and out of tomato and pepper roots was described by polynomial and linear models, respectively (Figure 6-10). Rimsulfuron content in the roots is maximal at 24 HAT in both species, but translocation into pepper roots was more rapid 24 HAT as compared to tomato. However, the difference in translocation rate is minor (1.3 vs.1.9 pmoles/mg dwt./hr). The content of rimsulfuron in the roots drops in tomato and pepper, 70 and 80%, respectively beyond 24 HAT. The uptake of rimsulfuron between 24 and 72 HAT is constant in pepper in contrast to an exponential increase in tomato. Rimsulfuron content in the roots also increases exponentially. Movement of absolute rimsulfuron content in the roots was similar to what was observed with the content of rimsulfuron/mg dwt., therefore the data was not presented.

The content of rimsulfuron/mg dwt. in tomato and pepper shoots increased with respect to time in a hyperbolic and linear pattern, respectively (Figure 6-11). Rimsulfuron translocation into tomato shoots was more rapid 24 HAT compared with pepper (0.5 vs. 0.08 pmoles/mg dwt./hr, resepctively). From 24 to 72 HAT, the rate of rimsulfuron accumulation in the shoots of tomato decreased.

The absolute content of rimsulfuron in tomato and pepper shoots was similar to the rimsulfuron/mg dwt. of plant, so absolute Bq data will not be presented either. The absolute content and rimsulfuron/mg dwt. of plant consistently increases over 72 hours in tomato shoots. During the same period the levels of rimsulfuron in the roots decreases 24 HAT, while uptake into the roots increases. The decreasing rimsulfuron levels in the roots is associated with the increasing translocation of the herbicide to the shoots. The rimsulfuron decrease in the roots is not proportional to the increase in the shoots. Furthermore, rimsulfuron levels in pepper (absolute and rimsulfuron/mg dwt. of plant) decrease in the roots and shoots over time. The herbicide was not eluted back into the vial, so the herbicide destination in these species is not well understood.

The 72 HAT autoradiographs show pepper has a darker root area (more radioactivity and herbicide) than tomato (Picture 6-2). Furthermore, the tomato is darker in the shoots, compared with pepper. These observations indicate that tomato has more rimsulfuron in the shoots and less herbicide in the roots as compared to pepper. This supports the conclusions drawn when the absolute rimsulfuron content was quantified 72 HAT in tomato and pepper. The dispersion of rimsulfuron in each section appears to differ between the species. The autoradiographs show more rimsulfuron is found in the shoot tips of tomato than pepper. However, the herbicide has left the vascular elements in both species.

Root absorption and translocation between tomato and pepper were quantified in these experiments. The results show that the amount of herbicide absorbed and sections translocated to are similar between tomato and pepper. Therefore tomato tolerance to preemergence applications of rimsulfuron is not a function of limited herbicide uptake or it being sequestered after absorption and not reaching the target site.

Tomato and pepper root uptake. Root uptake of halosulfuron in tomato and pepper was between 14 to 30% in the two experiments. Root uptake of rimsulfuron in tomato and pepper was 30 and 13%, respectively. The amount of halosulfuron and rimsulfuron taken up appears to be similar, but the mechanism of uptake may differ. Tomato and pepper exhibited linear and hyperbolic uptake of halosulfuron in both experiments. It is difficult to determine if halosulfuron uptake in these species is passive or active, based on the results. Further studies using varying concentrations and metabolic inhibitors will be needed to confirm the mechanism of uptake. Evidence that sulfonylureas root uptake is an active process in some species does exist. Chlorimuron absorption in velvet leaf is hyperbolic with respect to time (Nandihalli and Bhowmik, 1989). Furthermore, root uptake of chlorimuron in velvetleaf was limited by metabolic inhibitors and anoxia.

No studies on root absorption and translocation of halosulfuron or rimsulfuron could be discovered in the literature. Comparisons of primisulfuron and nicosulfuron uptake in sugar beet to halosulfuron and rimsulfuron uptake in tomato and pepper, exhibit the higher absorption in the latter. Uptake of nicosulfuron and primisulfuron was 1 and 3%, respectively at the highest dose of 25 μg/L (Novosel and Renner, 1995).

Translocation patterns of rimsulfuron and halosulfuron were different in tomato and pepper. Tomato and pepper translocated a greater percentage of the absorbed rimsulfuron out of the roots as incubation time increased (up to 57% 72 HAT).

Halosulfuron translocation into tomato and pepper shoots always increased with time, but during the first experiment the percentage of halosulfuron absorbed in the plant translocated to the shoots decreased from 52% 24 HAT to 30% 72 HAT. In the second experiment, halosulfuron content in the shoots increased slightly over time from 20% 24 HAT to 25% 72 HAT. Translocation of halosulfuron following root absorption in tomato and pepper was comparable to nicosulfuron and primisulfuron translocation in sugar beets (Novosel and Renner, 1995). Translocation of nicosulfuron into sugar beets shoots was initially more rapid compared to primisulfuron, but was reduced over time (Novosel and Renner, 1995). The rapid increase in herbicide content in the shoots followed by steady decrease, is similar to what was observed with halosulfuron. This indicates is consistent with the possibility of early halosulfuron movement into the shoots via xylem, followed by steady phloem movement of herbicide back into the roots.

Regardless of the mechanism of halosulfuron or rimsulfuron transport, adequate levels were present in each section to inhibit ALS activity. In both experiments, roots of tomato and pepper contained similar amounts of halosulfuron 72 HAT (49 and 48 pmoles/mg dwt. in the first experiment and 131 and 116 pmoles/mg dwt. in the second). In each experiment, herbicide content in the shoots of the species were different (10 and 4 pmoles/mg dwt.). However, tomato and pepper ALS was severely inhibited by halosulfuron at 0.008pmoles in 400 μL of buffer (Chapter 4). Therefore, enough halosulfuron is present in both the roots and shoots to elicit a herbicidal response in these species. Tomato and pepper contained 31 and 46 pmoles/mg dwt. of rimsulfuron in the roots, respectively. ALS in these species was inhibited approximately 50% by 1.05pmoles of rimsulfuron. Again, the levels of herbicide (rimsulfuron) in each species is high enough to inhibit ALS and elicit a herbicidal response.

Foliar Uptake and Translocation

Halosulfuron. The foliar uptake data from the two halosulfuron experiments were pooled across time and species, since the time experiments were conducted did not interfere with halosulfuron uptake or the influence of species. The uptake of halosulfuron into tomato and pepper foliage over time occurs in a hyperbolic manner (Figure 6-12). Uptake is rapid in the first 24 HAT absorbing 40% of the applied herbicide (based on the model), with similar rates in both species. The rate of absorption in the first 24 HAT was 0.31 nmoles/hr in tomato and 0.25 nmoles/hr in pepper. After the first 24 HAT, the uptake would gradually increase up to 56% by 72 HAT.

Results from foliar halosulfuron translocation indicated that the time experiments were conducted influenced the treatment and segment area, therefore data were separated by experiment. In both experiments treatment time and segment area significantly affected halosulfuron movement in the plant. The data from both experiments were separated by segment area, then regressed against treatment time. The means from each species were graphed separately to demonstrate the similarities between them.

The content of halosulfuron/mg dwt. in the treated leaf increases over time, but plateaus between 24 and 72 HAT (Figure 6-13). Translocation of halosulfuron into the treated leaf was at a rate of 4.0 and 2.2 nmole/mg dwt./hr in tomato and pepper, respectively the first 24 HAT. All uptake data indicates that movement of herbicide into the plant plateaus between 48 and 72 HAT. Halosulfuron/mg dwt. data exhibited a pattern similar to the pattern of uptake where the content plateaus.

The translocation data of halosulfuron/mg dwt. into the rest of the plant indicated that there was little translocation of the halosulfuron away from the point of entry into the plant (Table 6-2). In most sections maximum translocation occurred at 48 HAT. Halosulfuron content in the sections of roots, above and below the treated leaf accounted for less than 0.1% of the radioactivity in the treated leaf. When this figure is adjusted to account for the labeled and unlabeled halosulfuron, then approximately 22 nmoles would enter the treated leaf 72 HAT. The maximum amount of halosulfuron translocated out of the treated leaf over 72hrs. was 0.34 pmoles/mg dwt. Most sections contained only 0.1 pmoles/mg dwt., therefore this data was not regressed.

In the second experiment, the translocation of halosulfuron/mg dwt. into the treated leaves was best described by polynomial models (Figure 6-14), which is similar to the first experiment (Figure 6-13). The rate of halosulfuron translocation into tomato and pepper leaves in the second experiment differed from the first experiment. Halosulfuron was translocated in pepper at a rate of 0.51 nmoles/mg dwt./hr compared with 0.34 nmoles/mg dwt./hr in tomato.

When the data was adjusted to account for plant weight, there was no detectable translocation. Since essentially no halosulfuron was translocated up into meristematic regions, movement into the upper shoots probably has little effect on the whole plant tolerance. The movement of absolute and halosulfuron/mg dwt. into the lower shoots was also not significant over time. As with translocation to the upper shoots, minimal amounts of halosulfuron was translocated to the lower portions of the plant (0.8% of halosulfuron in the plant) (Table 6-3).

Little halosulfuron could be detected in the roots of plants. Similar to what was observed in other segments of the plant, the maximum halosulfuron content in the roots was detected between 48 and 72 HAT. The most halosulfuron found in the roots was 0.8% of the detectable halosulfuron (Table 6-3).

The qualitative data is in agreement with quantitative data, showing no difference between the species (Picture 6-3). The autoradiographs do not show an appreciable translocation of the herbicide to the upper shoots. The findings of the autoradiographs corroborate the quantitative data from the liquid scintillation counter. There was detectable halosulfuron in the lower shoots, which was slightly higher in pepper than tomato. These findings are in agreement with the data obtained through oxidation, where only 0.8% of the herbicide absorbed translocated to the lower shoots and roots.

Rimsulfuron. The time the experiment was conducted significantly affect the foliar uptake of rimsulfuron (treatment), the response of each species to the treatment, and the interaction between species and treatment. The uptake data was separated by experiment and each experiment was further analyzed. In the first experiment, the treatment and species significantly affected foliar uptake. The species did not significantly interact with the treatment, so data from each species was pooled and regressed against time (Figure 6-15). The uptake of rimsulfuron was rapid from 0 to 24 HAT (0.56 nmoles/hr). After this time (24 HAT) the uptake in both species was gradual between 24 and 72 HAT. Maximum uptake of rimsulfuron in pepper and tomato was 87 and 80% of rimsulfuron applied, respectively 72 HAT (Figure 6-15).

In the second experiment, the species significantly affected the uptake of the herbicide, but treatment did not influence the uptake or species affect on rimsulfuron uptake. Tomato uptake of rimsulfuron in the first and second experiments were similar. Maximum uptake was 80% 72 HAT in both experiments with an overall gradual (14%) increase in uptake from 24 to 72 HAT. Absorption of rimsulfuron in pepper was nominal in the second experiment up to 48 HAT. Between 48 and 72 HAT there was a 18% increase in the uptake of rimsulfuron from 0 HAT. The increased uptake between 48 and 72 HAT indicates the plants were viable. The metabolic activity of pepper may have been different in each experiment, but the level of metabolic activity was not quantified.

The translocation of rimsulfuron absorbed into tomato and pepper was investigated to determine its influence on whole plant tolerance. The time each experiment was conducted did not influence the results, so data from experiments was combined. The rimsulfuron/mg dwt. was then analyzed to determine the effect of treatment (time of incubation), the species, the plant section, and how combinations of each variable influenced the movement of rimsulfuron in the plants. The section was the only factor that was significantly influencing the rimsulfuron/mg dwt. in the plant. Therefore the data from each section was pooled across each time point. The two species were graphed separate to emphasize the similarities between them.

Rimsulfuron movement in tomato or pepper is minimal over time. Significantly more rimsulfuron was found in the area where it was applied than the middle or lower third section (Figure 6-16). Furthermore, over the entire experiment only 2.1 and 6% of the rimsulfuron was transported out of the treatment area in tomato and pepper, respectively.

Evaluation of the audioradiographs also show that little of the herbicide was transported out of the treated leaf (Picture 6-4). There is rimsulfuron in the stems of both tomato and pepper, but there is clearly more in pepper which supports the quantitative findings. Furthermore, no herbicide appears to be in the root tissue, which also corroborates the quantitative findings. This data provides evidence phloem movement of rimsulfuron is minimal in tomato and pepper.

Tomato and pepper foliar uptake

Foliar applications of sulfonylureas typically result in 11% of the material being absorbed (Wittenbach, 1994). Studies of foliar uptake of halosulfuron and rimsulfuron in tomato and pepper revealed much higher absorption rates. Uptake of halosulfuron and rimsulfuron was ~60 (15 nmoles) and 80% (9 nmoles), respectively, in tomato and pepper after 72 hours. There are few published studies investigating uptake and/or translocation of either rimsulfuron or halosulfuron. Compared with reported uptake of these herbicides in other species, halosulfuron uptake in purple nutsedge was higher than what was observed with tomato, or pepper (Khan and Molin, 2001). Rimsulfuron uptake is similar to what was reported in other species (Ackley et al., 1999; Mekki and Leroux, 1994; Brown et al., 1991).

Purple nutsedge, which is susceptible to halosulfuron, absorbed nearly 100% of the halosulfuron applied within 24 hours (Khan and Molin, 2001). Khan and Molin

(2001) did not indicate the pattern of halosulfuron uptake exhibited in purple nutsedge. With nearly 100% of applied halosulfuron being absorbed, active uptake in purple nutsedge is assumed. Uptake in tomato and pepper began to plateau 48 HAT, indicating saturation of an active uptake process (Figure 6-12). No other examples of halosulfuron uptake in plants is available to compare with the findings reported here. However, differential foliar uptake of a sulfonylurea in different plant families is common. Florida beggarweed, common cocklebur, sicklepod, and yellow nutsedge are sensitive to chlorimuron, and absorb 11 to 85% from foliar applications (plants were oversprayed with 5.3 g ai ha^{-1} and spiked with 13 nmoles of ^{14}C chlorimuron) (Wilcut et al., 1989). Foliar absorption of triflusulfuron in sugar beets, lambsquarter, and wild mustard was reported between 6 and17% (Wittenbach, 1994). Thifensulfuron uptake through the foliage of soybeans, redroot pigweed, lambsquarter, and velvetleaf was between 10 to 48% (5 to 24 nmoles) depending on the species (Brown et al., 1994). Furthermore, differential uptake and translocation of a herbicide have been observed in plants from the same family. Ethoxysulfuron uptake in wheat and rice was 18 and 37%, respectively while rice translocated ½ the amount wheat did (0.2 versus 0.4%) (Kocher and Dickerhof, 1995).

Foliar uptake of rimsulfuron in tomato and pepper was similar to the uptake reported in nightshade species (*Solanum spp.*), *Panicum miliaceum*, and *Digitaria ischaemum*. Uptake of rimsulfuron in hairy (*Solanum sarrachoides*), eastern black (*Solanum ptycanthum*), black nightshade (*Solanum nigrum*), *P. miliaceum*, *D. ischaemum*, *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, and *Avena fatua* was rapid within 24 HAT, and leveling off after this time (Ackley et al., 1999; Mekki and Leroux, 1994). These three nightshade species absorbed between 54 to 74% of the applied ^{14}C rimsulfuron (2.5 to 3.5 nmoles) 72HAT, while the other five species absorbed 20 to 45% (0.4 to 0.9 nmoles) within 48 HAT. The total amount and pattern of rimsulfuron uptake in tomato and pepper was similar to what was observed in the nightshade species. Tomato and pepper absorbed approximately 74 to 84% more ^{14}C rimsulfuron than the *P. miliaceum*, *D. ischaemum*, *A. retroflexus*, *A. artemisiifolia*, and *A. fatua*.

Furthermore a common uptake pattern of sulfonylureas in solanaceous species appears to exist. The majority of chlorsulfuron and rimsulfuron uptake in eastern black nightshade occurs within 24 HAT, with uptake plateau after this time (Ackley et al., 1999; Hageman and Behrens, 1984). The same patterns of rimsulfuron uptake was observed with hairy and black nightshade. Halosulfuron and rimsulfuron uptake in tomato and pepper was also rapid in the first 24 HAT, with the rate of uptake decreasing after this time (Figure 6-12 and 6-15).

Low amounts (<1% or 0.2 nmoles) of halosulfuron was translocated out of the treated leaf in tomato and pepper. Translocation to all plant sections increased over time. Halosulfuron translocated equivalently to sections in purple nutsedge within 24 HAT. After 24 hours, halosulfuron levels were static. The percentage of halosulfuron translocated out of the treated leaf was not reported, so no comparisons with tomato or pepper can be drawn. However, it appears the translocation that occurs in purple nutsedge is more rapid than tomato or pepper. Low translocation of halosulfuron has also been reported in maize following foliar application (Logusch et al., 1998). Logusch et al.

(1998) did not report the rate or mechanism of halosulfuron translocation, therefore patterns of translocation could not be compared. It appears that halosulfuron does not move to a great extent in cultivated plants after absorption.

Translocation of rimsulfuron was limited in tomato and pepper, 4 (0.3 nmoles) and 11% (0.8 nmoles), respectively. The translocation of tomato in this study is lower than the translocation reported in 'Mountain Pride' tomato or in some weed species. Ackley et al. (1999) reported almost 50% rimsulfuron translocation of the 4.7 nmoles of ^{14}C rimsulfuron applied within 24 HAT for each species (hairy, eastern black and black nightshade). This is similar to what Robinson et al. (1997) reported in 'Mountain Pride' tomato (46%). Mekki and Leroux (1994) reported 15 to 30% (0.06 to 0.3 nmoles) translocation out of treated leaves 24 HAT in *P. miliaceum*, *D. ischaemum*, *A. retroflexus*, *A. artemisiifolia*, and *A. fatua*. Rimsulfuron translocated out of nightshades leaves moved primarily to the upper sections of the plants. Translocation of chlorsulfuron out of eastern black nightshade leaves was 80% 48 HAT, and also ended up in leaves above the treated leaf. Mekki and Leroux (1994) reported rimsulfuron primarily translocated to the upper shoots of dicots, which was the opposite for monocots. The indication of these studies is that the majority of the herbicide absorbed in dicots moves into the upper shoots.

Brown et al. (1991) reported limited translocation of rimsulfuron following foliar applications to maize and potato. Rimsulfuron movement in tomato, pepper, and potato was minor and equally distributed. Findings by Brown et al. (1991) are in contrast to the movement of rimsulfuron and chlorsulfuron in weeds (Ackley et al., 1999; Mekki and Leroux, 1994; Hageman and Behrens, 1984).

Translocation of halosulfuron and rimsulfuron in crop plants appears to be minimal, but it does occur through both the xylem and phloem (Logusch et al., 1998; Brown et al., 1991). The levels of halosulfuron and rimsulfuron that enters tomato and pepper is high enough to elicit a herbicidal response. Halosulfuron content in treated leaves was estimated based on recommended use rates used in the experiment and uptake data (Figure 6-12). Halosulfuron content of the treated leaf in the first experiment was 9 to 14 nmoles in tomato and pepper, respectively. In the second experiment the content was estimated to be 1 to 1.5 nmoles. ALS of tomato and pepper was inhibited by halosulfuron levels 1,000 times lower.

A similar situation is observed with foliar applications of rimsulfuron. Rimsulfuron is readily absorbed by both species, which significantly influences the whole plant response. Based on the recommended field rates used and the uptake data (Figure 6-15) approximately 5 to 6 μmoles of rimsulfuron would be expected in each leaf treated. Rimsulfuron levels this high would be 1,000,000 times higher than needed for 50% inhibition of ALS (Chapter 4).

Quantitative data from uptake and translocation experiments demonstrate that equivalent amounts of herbicide are absorbed and transported in tomato and pepper. Furthermore, the initial rate of translocation was usually similar in tomato and pepper. Qualitative data demonstrates the herbicide is dispersed similarly in both species. When this data is considered with ALS inhibition data from Chapter 4, then the roles of limited uptake and/or sequestration as mechanism of tolerance are minimized in importance.

Table 6-1. Average stomatal density of adaxial leaf surface of tomato and pepper.

	Tomato	Pepper
$\bar{x}$ Stomatal Density Greenhouse	20.6 ± 4.54	17.6 ± 3.85
$\bar{x}$ Stomatal Density Field	16.2 ± 3.27	17.8 ± 9.25

Table 6-2.Halosulfuron dwt. (Bq/mg dwt.) found in tomato and pepper sections during experiment 1.

	Tomato		
HAT	Root	Lower Shoot	Upper Shoot
0	0.0024	0.0045	0.0029
24	0.0041±0.0021	0.0141±0.0083	0.0089±0.003
48	0.0035±0.0022	0.00213±0.0076	0.0167±0.0101
72	0.0028±0.0028	0.0144±0.0011	0.0098±0.0052
HAT	Pepper		
0	0.00075	0.0075	0.0029
24	0.0013±0.0005	0.0173±0.0108	0.0167±0.007
48	0.0033±0.0016	0.0167±0.0021	0.0129±0.001
72	0.0023±0.0014	0.0115±0.0031	0.0151±0.0041

Table 6-3. Halosulfuron/mg dwt. (Bq/mg dwt.) found in tomato and pepper sections during experiment 2.

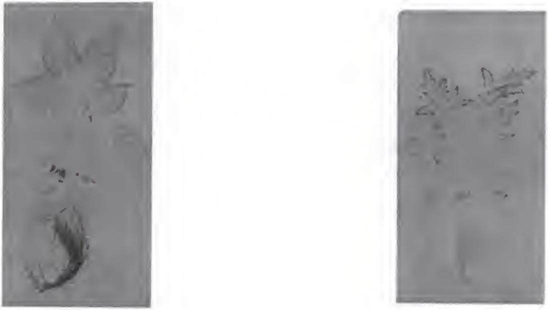
	Tomato		
HAT	Root	Lower Shoot	Upper Shoot
0	0.0016	0.0056	0.011
24	0.0015±0.0008	0.0041±0.0013	0.0088±0.0003
48	0.0014±0.0003	0.0033±0.0021	0.9±0.73
72	0.0118±0.21	0.0325±0.0381	0.9±0.82
HAT	Pepper		
0	0.0007	0.0031	0.0123
24	0.0007±0.00003	0.0095±0.0038	0.0083±0.0003
48	0.0118±0.0119	0.0075±0.0057	0.0097±0.00249
72	0.0038±0.0007	0.0173±0.0078	0.0083±0.0031

Picture 6-1. Autoradiograph of plants supplied with halosulfuron to roots and allowed to translocate for 72 HAT.

Pepper

Tomato

Picture 6-2. Autoradiograph of plants supplied with rimsulfuron to roots and allowed to translocate for 72 HAT.



Pepper

Tomato

Picture 6-3. Halosulfuron foliar uptake and translocation experiment 72 HAT.

Pepper

Tomato

Picture 6-4. Rimsulfuron foliar uptake and translocation experiment 72 HAT.

Pepper

Tomato

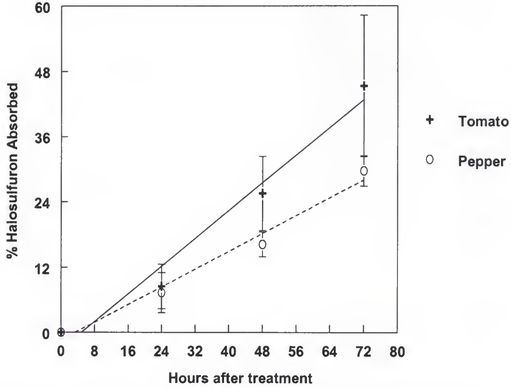


Figure 6-1. Tomato and pepper root uptake of halosulfuron in Experiment 1. Uptake in tomato=-3.1+0.64x r^2=0.98. Uptake in pepper =-1.38+0.41x r^2=0.99.

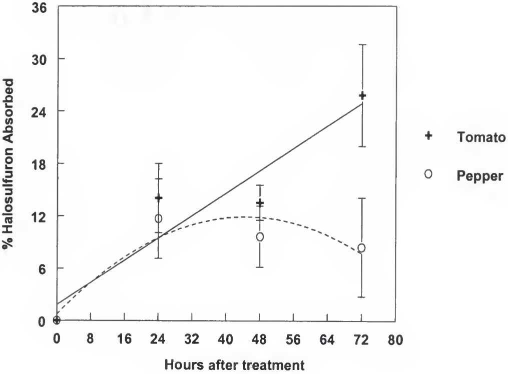


Figure 6-2. Tomato and pepper root uptake of halosulfuron in Experiment 2. Uptake in tomato= 1.82+0.32x r^2=0.94. Uptake in pepper=0.73+0.50x+-0.0056x^2 r^2=0.93.

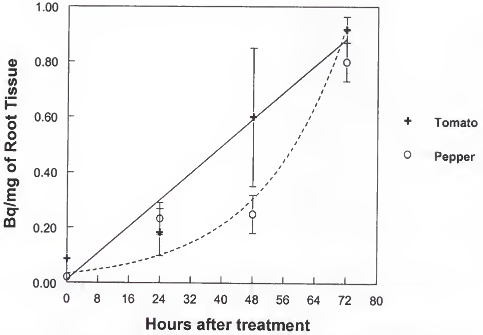


Figure 6-3. Translocation of halosulfuron into and out tomato and pepper roots in Experiment 1.Bq/mg dwt. of tomato= 0.01+0.01211x r^2=0.97 and Bq/mg dwt. of pepper= 0.032+exp0.0464x r^2=0.92.

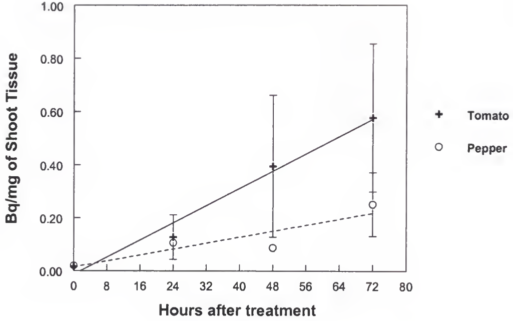


Figure 6-4. Halosulfuron translocation into the middle ⅓ of tomato and pepper plants in Experiment 1.Bq/mg dwt. of tomato= -0.013+0.0081x r^2=0.98 and Bq/mg dwt. of pepper= 0.015+0.0028x r^2=0.89.

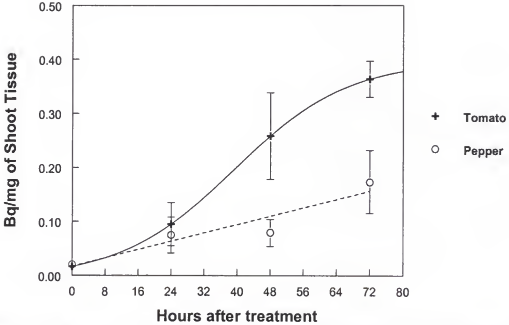


Figure 6-5. Halosulfuron translocation into the upper ⅓ of tomato and pepper plants in Experiment 1. Bq/mg dwt. in tomato= -0.0054+0.4/(1+exp(-(x-39.17)/13.7)) r^2=0.99 Bq/mg dwt. in pepper=0.017+0.0019x r^2=0.94

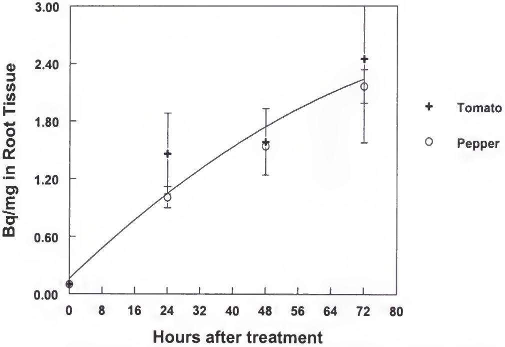


Figure 6-6. Translocation of halosulfuron into and out tomato and pepper roots in Experiment 2. $Y= 0.16+0.041x+-0.00016x^2$ $r^2=0.98$

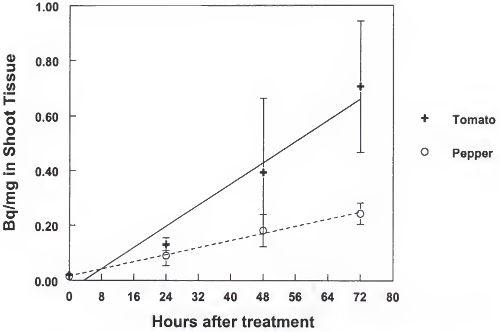


Figure 6-7. Halosulfuron translocation into the middle ⅓ of tomato and pepper plants in Experiment 2. Bq/mg of tomato= -0.035+0.0096x r^2=0.98, and Bq/mg dwt. of pepper= 0.016+0.0032x r^2=0.99.

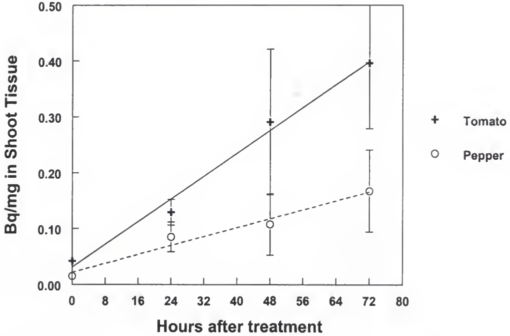


Figure 6-8. Halosulfuron translocation into the upper ⅓ of tomato and pepper plants in Experiment 2. Bq/mg dwt. of tomato= 0.031+ 0.005x r^2=0.99, and Bq/mg dwt. of pepper= 0.021+0.002x r^2=0.98

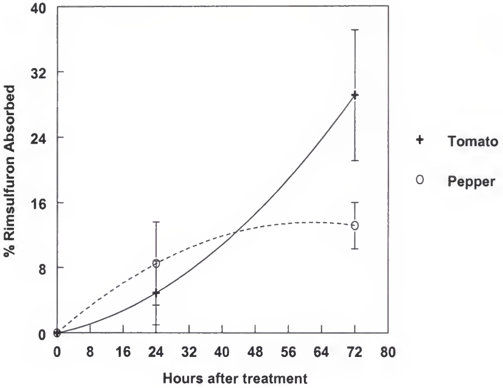


Figure 6-9. Rimsulfuron root uptake in tomato and pepper. Tomato= -4.9×10^{-15} + $0.104x + 0.0042x^2$ r^2=0.99 and pepper=-5.82×10^{-15} + $0.44x + 0.0036x^2$ r^2=0.99.

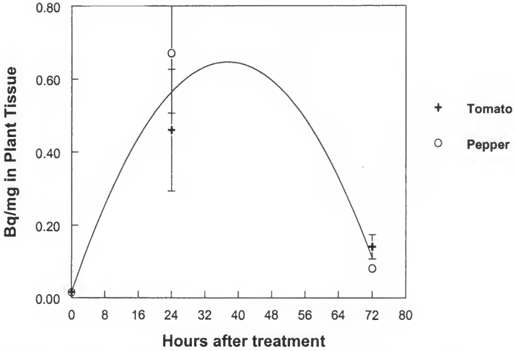


Figure 6-10. Rimsulfuron content in tomato and pepper roots. Y= 56 + 119x + -1.59x^2 r^2=0.98

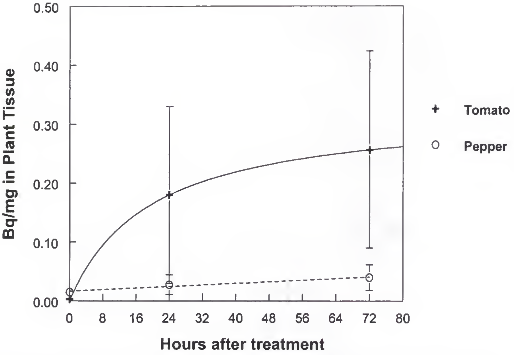


Figure 6-11. Rimsulfuron content in shoots of tomato and pepper. Bq/mg dwt. of tomato= 0.0325*0.0168*x/(0.0325+0.0168*x) r^2=0.99, and Bq/mg dwt. of pepper= 0.0169+0.0003x r^2=0.98.

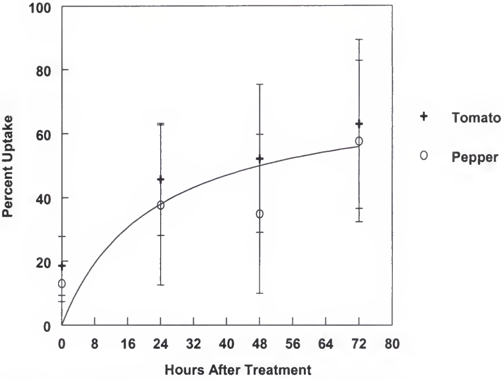


Figure 6-12. Halosulfuron foliar uptake in tomato and pepper. Y=72.9*3.3*x/(72.9+3.3*x) r^2=0.88.

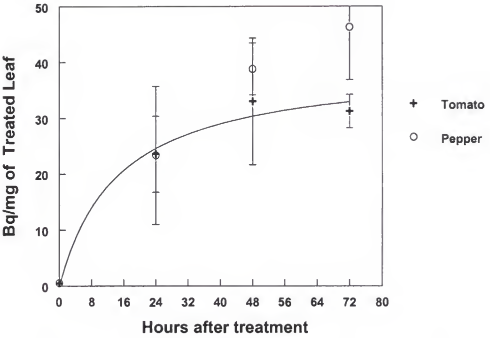


Figure 6-13. Content of halosulfuron/mg dwt. in treated leaf segments of tomato and pepper during experiment 1. Y=39.6*2.69*x/(39.6+2.69*x)r^2=0.99

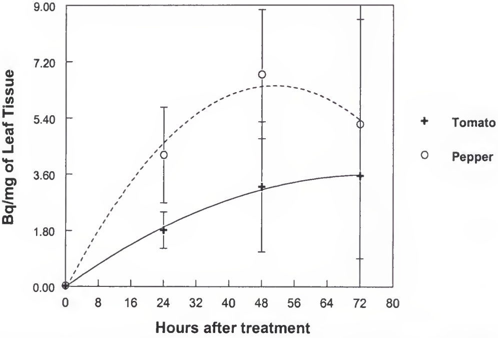


Figure 6-14.Content of halosulfuron in treated leaf of tomato and pepper during experiment 2. Bq/mg dwt. of tomato= -0.011+0.095x+-0.0006x^2 r^2=0.99, and Bq/mg dwt. of pepper= -0.09+0.255x+ -0.0025x^2 r^2=0.99.

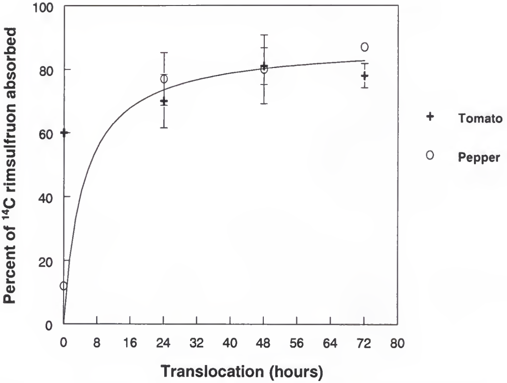


Figure 6-15. Rimsulfuron foliar uptake in tomato and pepper. Y=88*18*x/(88 + 18*x) r^2=0.79

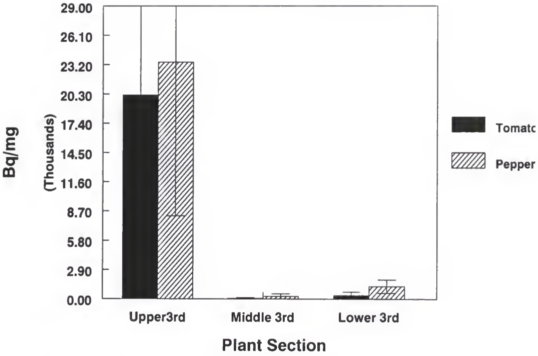


Figure 6-16. Rimsulfuron content/mg dwt. of tomato and pepper segments.

CHAPTER 7
METABOLIC TRANSFORMATIONS OF RIMSULFURON IN TOMATO (*Lycopersicum esculentum* Mill.) AND PEPPER (*Capsicum annum* L.)

Introduction

The most common mechanism of herbicide tolerance in plants is through transformation of the herbicidal compound into non-phytotoxic metabolites. The non-phytotoxic metabolites are typically formed through biological processes. Usually the biological process is a metabolic transformation controlled by one of two classes of enzymes [cytochrome P-450s (CYP-450s) or glutathione-S-transferases (GSTs)] (McGonigle et al., 1997; Schuler, 1996).

All of the sulfonylureas (SU) used commercially have been shown to undergo biological transformation in at least one species of plant (Roberts et al., 1998). The transformation mechanisms of a given SU is not the same in each plant species (Koeppe and Brown, 1995). O-demethylation and hydroxylation are usually the initial steps of transformation reported in plants. After these two initial transformations the compound is typically conjugated, followed by bridge contractions and/or cleavage (Roberts et al., 1998). O-demethylation and hydroxylation are typically controlled by the CYP450s (Schuler, 1996; Hatzios, 1994).

Rimsulfuron is also transformed in plants (Koeppe et al., 2000; Brown et al., 1991). The sulfonylurea bridge of rimsulfuron undergoes rearrangement/contraction in maize. Koeppe et al. (2000) indicated the bridge contraction and rearrangement was abiotic, but hydrolysis of the pyrimidine ring followed by glucose conjugation was an enzymatic process.

Biotic transformation of herbicides have been documented in tomato and pepper. Metribuzin, napropamide, and diphenamid are transformed into non-phytotoxic metabolites ultimately through conjugation with carbohydrate moieties (Frear et al., 1983; Hodgson et al., 1973). Pepper will metabolize clomazone and diphenamid. Diphenamid metabolism is approximately nine times greater in tomato than pepper, but nonetheless pepper is more tolerant (Weston and Barrett, 1989; Hodgson and Hoffer, 1977; Hodgson et al., 1973).

Materials and Methods

Plants used in metabolism studies were grown in a greenhouse under natural lighting. Plants used in these studies were between 30-40 days old, with 6-8 true leaves. Shoot explants from tomato and pepper were cut under water at the juncture of the stem and petiole. Explants were then transferred to tapered vials containing 4 mL of aqueous rimsulfuron solutions, buffered with 2 mM of potassium-phosphate (pH 7.0). Rimsulfuron solutions were allowed to absorb into explants for six hours. When vial volumes dropped below 500 μL, they were refilled with solutions of buffer only. After the six hour uptake period the explants were transferred to solutions containing only buffer for eighteen hours. Treatments of each experiment were explants of tomato and pepper incubated for 24 hours in a buffer solution with no herbicide (control), herbicide added at immediately proceeding extraction [spike treatment (to monitor abiotic breakdown)], and a ^{14}C rimsulfuron treatment (tracer). Each 24 hour incubation containing the non ^{14}C labeled rimsulfuron was replicated twice. All other treatments were replicated once.

Solutions containing rimsulfuron, supplied to tomato and pepper, were of a technical grade (>95% pure) quality. Rimsulfuron solutions were prepared by mixing 0.011 g in 42 mL of an autoclaved 2 mM potassium-phosphate buffer (pH 7.0). Four mL from this stock was equivalent to 0.963 μmoles. The treatment containing non and ^{14}C labeled rimsulfuron was made by adding 0.963 μmoles as previously described and 1.17 nmoles of ^{14}C rimsulfuron provided by New England Nuclear Sciences (lot# 3110-241A). The herbicide was 95.7% pure with a specific activity of 1.68 MBq/mg.

Once explants were transferred into vials they were randomly placed on an orbital shaker set at 500 rpm. The experiment was conducted under continuos artificial lighting (56 μmole s^{-1}). Temperatures of each experiment were between 25 and 29° C, because optimum metabolism in maize shoots occurs between 25 and 30° C (Koeppe et al., 2000).

At the end of each incubation period plants were removed from vials and prepared for extraction, by placing each leaf in a petri dish and weighing it. Extraction of rimsulfuron and metabolites were carried out immediately after each incubation period. All glassware used in the extraction procedure was acid washed for 24 hours prior to use. The solvent used in the extraction procedures was acetonitrile:water based (80:20 v/v). The solvent was buffered with 5 mM of a potassium-phosphate buffer pH 7.0. The buffer was made with autoclaved distilled water. Extraction efficiency of rimsulfuron and its metabolites were monitored by taking a 100 μL subsample of leaves treated with ^{14}C rimsulfuron counting the radioactivity using a liquid scintillation counter (LSC). Radioactivity discovered in 100 μL was extrapolated out to the total volume of solvent in ^{14}C rimsulfuron tomato and pepper treatments.

After extraction rimsulfuron and metabolites were initially resolved from samples through the use of thin layer chromatography (TLC). Rimsulfuron and metabolites were identified using autoradiography and specific regions were extracted from TLC plates in acetonitrile (Appendix H). Volume was reduced for further analysis. Metabolites were separated using high performance liquid chromatography (HPLC) and the peaks were detected using a ultraviolet light (UV) detector set at 525 nm. Rimsulfuron was initially identified based on comparison of the retention time with standard rimsulfuron. Rimsulfuron was further analyzed using electro-spray ionization (+ESI) mass spectrometry (MS) to confirm the identity.

Results and Discussion

The experiments described above were repeated twice. In each experiment the level of rimsulfuron in each extract was quantified using an external rimsulfuron standard made from the technical grade (>95% pure). The different metabolites were described using their molecular weight, but without standards for each metabolite it was difficult to quantify the metabolites. The percentage of original rimsulfuron remaining in pepper after twenty four hours varied with each experiment. However, the percent of intact rimsulfuron remaining in pepper was never lower than tomato.

Rimsulfuron uptake between tomato and pepper was comparable. Tomato and pepper absorbed 98 and 80%, respectively, of the ^{14}C rimsulfuron supplied. The ^{14}C rimsulfuron was 0.001% of the total rimsulfuron supplied. ^{14}C rimsulfuron recovery in tomato and pepper was 72 and 87%, respectively. Rimsulfuron retention times of tomato and pepper were similar in the HPLC system used. In tomato rimsulfuron elution was 47 minutes after injection, while retention times in pepper were between 44 and 51 minutes (Figure 7-1 and 7-2). Identity of rimsulfuron was confirmed using (+) ESI-MS.

The uptake and recovery values were used to correctly quantify the μmoles of rimsulfuron supplied and remaining in each explant. The amount of rimsulfuron supplied and remaining in each species after twenty four hours is listed in Table 7-1. Tomato metabolized 42.6 nmoles of rimsulfuron/hour, which is approximately 8 times faster than the 5.7 nmoles/hour pepper metabolized.

Five different metabolites of rimsulfuron were discovered in tomato explants from experiment one. The metabolites had retention times of 23, 40, 59, 62, and 64 minutes and molecular weights (MW) of 250, 155, 198, 367, and 310, respectively (Figure 7-6 to 7-10). Four metabolites of rimsulfuron were discovered in extracts from pepper explants (Figure 7-6 to 7-10). The metabolites with (MW198 at 58 minutes, MW250 at 24 minutes, and MW367 at 63 minutes) in pepper were common to both tomato and pepper. Metabolites with MW of 155 or 310 were unique to tomato. Pepper did have a metabolite not found in tomato, with a retention time of 66 minutes and a MW of 324. When all of the UV area of a sample was considered, MW198 and 367 had the strongest UV signal of all the metabolites. These metabolites accounted for 45% of the UV area, while the other three metabolites accounted for less than 3%. Comparison between the metabolites is difficult, since no standards for each metabolite were available. Therefore, no correlation between the mole of each metabolite and UV signal could be made. If all metabolites gave equivalent UV signals, then based on abundance the MW367 metabolite is produced first, followed by MW198, MW310, and finally MW155 and MW250.

Rimsulfuron metabolites, with the MW of 367, 198, 250, 155, and 324 were described by Schneider et al. (1993). The MW 367 metabolite was formed through a loss of SO_2 in the sulfonyl ureas bridge which resulted in bridge contraction, and ultimately a MW324 metabolite. In the study by Schneider et al. (1993) the MW 198 metabolite was formed through a splitting of the sulfur bridge of rimsulfuron, which resulted in a loss of the C=O group. This reaction initially yields MW250 and 155 metabolites, from which MW198 is produced. These reactions were described as minor chemical breakdown reactions.

Rimsulfuron uptake in tomato was double what it was in pepper 24 HAT (62 vs.28%). The recovery of rimsulfuron and metabolites was good in both species (>82%). The data used to compare between tomato and pepper was adjusted for uptake and recovery. In the second experiment, the retention times of rimsulfuron and metabolites were similar between species and similar to the first experiment. Rimsulfuron entered the mobile phase 47.7 minutes after injection, similar to the 47.9 minutes required with pepper (Figure 7-3 and 7-4). When the rimsulfuron stock was analyzed by itself, it entered the mobile phase 45.5 minutes after injection. In the second experiment the identity of rimsulfuron and metabolites were also confirmed using (+)ESI-MS. Tomato metabolized 0.418 μmoles of rimsulfuron in 24 hours or 17.4 nmoles/hr. Pepper metabolized 0.008 μmoles in 24 hours or 0.33 nmoles/hr (Table 7-2). Rimsulfuron is metabolized at a rate 52.3 times faster in tomato than pepper. The difference between the species in rate of metabolism was much higher in the second experiment compared with the first. In the second experiment both tomato and pepper had similar metabolites. The metabolites had MW of 198, 310, 324, and 367 (Figure 7-6 to 7-10).

Differential rimsulfuron sensitivity between species has been reported in the past. Robinson et al. (1997) reported tomato metabolized rimsulfuron while eastern black nightshade did not. However, there was no indication of the percentage of rimsulfuron metabolized in each species or what the metabolites might be. Koeppe et al. (2000) reported a at least a 40% difference between rimsulfuron sensitive and tolerant species. In these experiments, tomato metabolized 80 to 98% more rimsulfuron than pepper.

Rimsulfuron metabolites identified in experiments with tomato and pepper were compared with metabolites formed in maize. The MW of the rimsulfuron metabolites found in tomato and pepper had the same MW as the metabolites that were formed through abiotic transformations in maize (Koeppe et al., 2000). In experiment one, rimsulfuron was added to tomato and pepper leaves just before extraction process began and maximum breakdown observed was 14 and 22%, respectively.

There is evidence that the transformations int tomato are at least enhanced through biological activity. From the stock solution, 2% of the rimsulfuron was broken down during a 6 hour incubation in the potassium-phosphate buffer (pH 7.0). Therefore, as a result of the extraction process, approximately 12 to 20% of the rimsulfuron is broken down in tomato and pepper, respectively. It is possible the breakdown observed during the 24 hour incubation of experiment one may be a result of chemical degradation and not an enzyme directly metabolizing the rimsulfuron. However, the biological activity of tomato and pepper enhances the rate of this breakdown, since rimsulfuron breakdown increased 80% when taken up in tomato leaves for 24 hours (data not shown). Furthermore, when rimsulfuron was absorbed into tomato leaves the breakdown increased 90% compared to rimsulfuron incubating in buffer alone for 24 hours (data not shown). The biological activity of tomato enhances rimsulfuron breakdown to a much greater extent as compared to pepper (Table 7-1). Koeppe et al. (2000) referred to the chemical reaction that produces the MW367 rimsulfuron derivative as a *ipso* bridge contraction. It takes 7.2 days, at pH 7.0, to convert half the rimsulfuron through this reaction to the MW367 derivative (Schneider et al., 1993). Rimsulfuron is almost completely metabolized in tomato (63 to 93%) and maize (100%), within 24hours. The metabolism in maize is an enzymatic process (Koeppe et al., 2000). When the data presented here is compared with examples of an enzymatic rimsulfuron metabolism, then parallels can be drawn that points to the enzymatic breakdown in tomato.

Tomato and pepper leaves were traced onto Whatman #5 filter paper, and the paper was cut into the shape of the leaf, and weighed. Then leaves were sprayed with 0.035 kg ai/ha of rimsulfuron with 0.25% v/v of a non-ionic surfactant at 270 L/ha. From this rimsulfuron quantity on tomato and pepper leaves were estimated to be 18.6 and 6.88

nmoles, respectively. Assuming 80% foliar uptake (Chapter 5), 14.9 and 5.5 nmoles would enter tomato and pepper leaves, respectively. If the substrate (pyruvate) levels were saturating (>20 mM) in tomato, then 0.02 pmoles of rimsulfuron would reduce ALS activity 50% (Chapter 4). Under the same assumptions, pepper ALS would be reduced 50% by 0.2 pmoles (Chapter 4). Therefore, ample rimsulfuron would enter the cell and cause a herbicidal effect. In the first experiment rimsulfuron was metabolized at a rate of 42.6 and 5.6 nmoles/hour in tomato and pepper, respectively. In the second experiment, rimsulfuron was metabolized at a rate of 17.4 and 0.33 nmoles/hr in tomato and pepper, respectively. If rimsulfuron breakdown occurred at these rates, with 14.9 nmoles entering tomato and 5.5 nmoles entering pepper, then both species would be expected to tolerate rimsulfuron applications. Since pepper cannot tolerate foliar rimsulfuron applications, then the breakdown, whether chemical or enzymatic, must occur at a reduced rate in pepper when only 6 nmoles is present. Chemical and enzymatic reactions can be influenced by concentrations of reactants or substrates, and this data does not clearly point to what controls rimsulfuron metabolism in tomato or pepper.

The data presented on rimsulfuron metabolism in tomato and pepper demonstrate that tomato metabolizes rimsulfuron at a faster rate than pepper. Hence, the differential metabolism is suggested to be the physiological basis for differential herbicide tolerance. The breakdown of rimsulfuron and other sulfonylureas not studied here have been suggested to be controlled by CYP-450, and the same enzymes are thought to participate in tomato as well (Roberts et al., 1998; Koeppe and Brown, 1995).

Table 7-1. Rimsulfuron content in tomato and pepper shoot explants from experiment 1, 24 hours after treatment.

	Tomato	Pepper
Rimsulfuron supplied	1.023μmoles	0.840μmoles
Rimsulfuron and metabolites recovered	0.737μmoles	0.731μmoles
Remaining intact rimsulfuron	0.052μmoles	0.641μmoles
Percent of rimsulfuron remaining	7.1	87.7

Table 7-2. Rimsulfuron content in tomato and pepper shoot explants from experiment 2, 24 hours after treatment.

	Tomato	Pepper
Rimsulfuron supplied	0.597μmoles	0.270μmoles
Rimsulfuron and metabolites recovered	0.490μmoles	0.270μmoles
Intact rimsulfuron remaining	0.179μmoles	0.262μmoles
Percent of rimsulfuron remaining	36.5	97.0

Figure 7-1. Elution profile of rimsulfuron and metabolites from experiment 1 tomato explants in HPLC system.

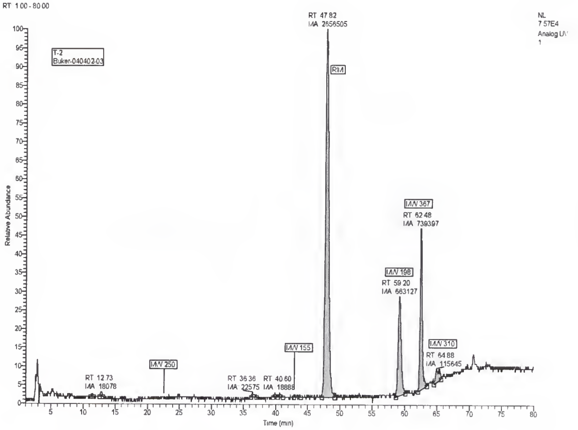


Figure 7-2. Elution of rimsulfuron and metabolites from experiment 1 pepper explants in HPLC system.

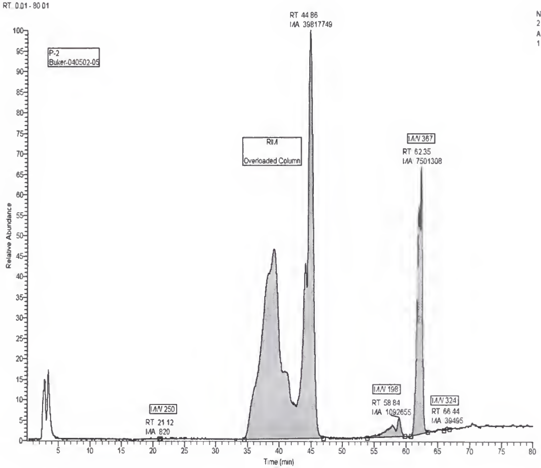


Figure 7-3. Rimsulfuron and metabolites from tomato explants during experiment 2 in HPLC system.

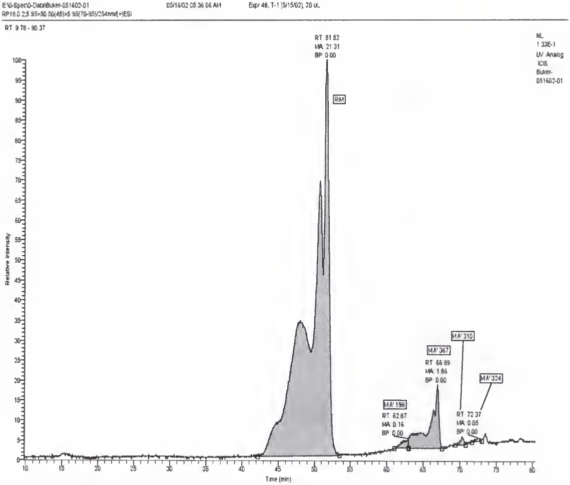
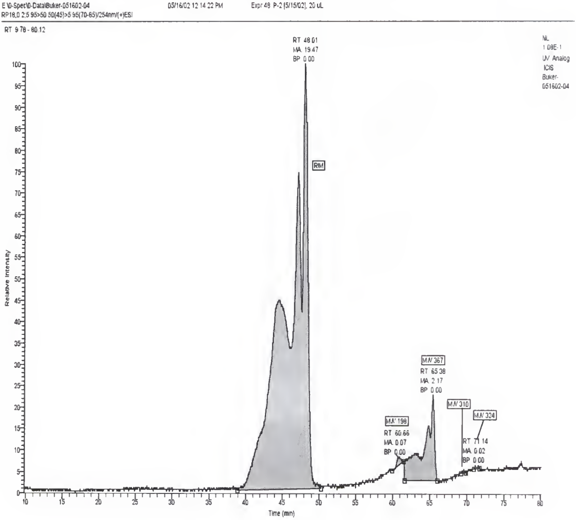


Figure 7-4. Elution of rimsulfuron and metabolites from pepper explants during experiment 2 in HPLC system.



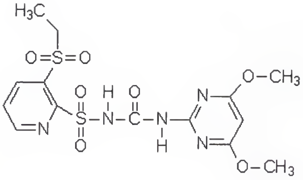


Figure 7-5. Rimsulfuron (MW413)

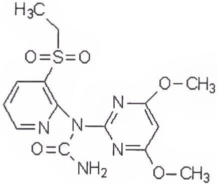


Figure 7-6. Metabolite 1 (MW367)

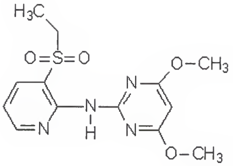


Figure 7-7. Metabolite 2 (MW324)

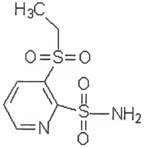


Figure 7-8. Metabolite 3 (MW250)

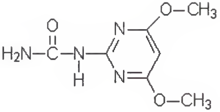


Figure 7-9. Metabolite 4 (MW198)

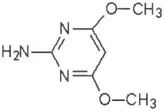


Figure 7-10. Metabolite 5 (MW155)

APPENDIX A
GREENHOUSE ENVIRONMENTAL CONDITIONS

Daily air temperature of greenhouse during first rimsulfuron and terbufos study.

Date	Maximum (°C)	Minimum (°C)
5/171999	44	23
5/19/1999	44	22
5/22/1999	42	21
5/24/1999	45	23
5/25/1999	45	24
5/26/1999	48	21
5/27/1999	47	22
5/28/1999	49	22
5/29/1999	49	24
5/30/1999	34	21
6/1/1999	34	21
6/3/1999	43	23
6/4/1999	44	21
6/6/1999	42	23
6/8/1999	44	22
6/10/1999	45	20

Daily air temperature of greenhouse during second rimsulfuron and terbufos study.

Date	Maximum (°C)	Minimum (°C)
6/22/1999	44	23
6/24/1999	44	22
6/25/1999	40	24
6/26/1999	39	24
6/27/1999	40	24
6/28/1999	39	24
6/29/1999	30	23
6/30/1999	38	22
7/1/1999	37	22
7/2/1999	44	22
7/6/1999	44	21
7/7/1999	45	24
7/8/1999	49	23
7/9/1999	43	23
7/10/1999	42	22
7/1299	43	23
7/3/99	47	25

Daily air temperature of greenhouse during first halosulfuron and terbufos study.

Date	Maximum (ºC)	Minimum (ºC)
9/2/2000	44	23
9/3/2000	42	16
9/4/2000	40	20
9/5/2000	39	20
9/6/2000	45	11
9/11/2000	42	15
9/12/2000	45	18
9/13/2000	44	16
9/16/2000	43	16
9/17/2000	41	14
9/22/2000	40	15
9/23/2000	37	17
9/24/2000	45	18

Daily air temperature of greenhouse during second halosulfuron and terbufos study.

Date	Maximum (°C)	Minimum (°C)
8/4/2001	45	24
8/5/2001	45	24
8/6/2001	45	22
8/7/2001	41	23
8/9/2001	42	24
8/10/2001	39	21
8/12/2001	46	24
8/13/2001	42	22
8/16/2001	43	23
8/17/2001	42	22
8/22/2001	43	21
8/23/2001	38	22
8/27/2001	44	21

Weekly air temperature of greenhouse during rimsulfuron and oxamyl study.

Date	Maximum (°C)	Minimum (°C)
8/4/2001	40	8
8/5/2001	44	10
8/6/2001	46	11
8/7/2001	45	11

Weekly air temperature of greenhouse during rimsulfuron and oxamyl study.

Date	Maximum (°C)	Minimum (°C)
8/4/2001	36	9
8/5/2001	41	8
8/6/2001	42	8
8/7/2001	44	4

Weekly air temperature of greenhouse during halosulfuron and oxamyl study.

Date	Maximum (°C)	Minimum (°C)
8/4/2001	36	9
8/5/2001	41	8
8/6/2001	42	8
8/7/2001	44	4

APPENDIX B
ALS EXTRACTION PROCEDURES

Part I Solutions for ALS Assay:

1. Sodium Pyruvate (Mwt. 110.05) was mixed at a concentration of 200mM (0.5502g / 25 mL dd H_2O) and stored in the refrigerator.
2. Thiamine Pyrophosphate [a.k.a cocarboxylase (Mwt. 460.8)] was mixed at a concentration of 5mM = 97% pure (0.0576g / 25 mL dd H_2O). This was prepared fresh before extraction and stored in a dark container at 4^0C.
3. FAD (Mwt. 785.56) 100 μM = 0.00784g / 100 mL dd H_2O. This was prepared fresh before extraction and stored in a dark container at 4^0C.
4. Protease inhibitors were mixed by adding 10 μM of leupeptin, 0.2mM 4-(2-aminoethyl) benzenesulfonyl fluoride, 1μM pepstatin A, 1μM bestatin, 1μM E-64, and 1mM1,10-phenanthroline together.

Part II Preparing Extraction Buffers

5. All tubes used in the extraction were pre-chilled and keep on ice.
6. Disposable Eppendorph® tubes were wrapped in aluminum foil and labeled "redissolve buffer."
7. DTT was removed from the freezer and placed in beaker with water to thaw.
8. Mortars were placed in the freezer to chill.
9. Redissolve mixture was prepared, with a final volume of 10 mL.

1M Phosphate buffer pH 7.0	1 mL	100 mM final concentration
$MgCl_2$ 6 H_2O 5mM	1 mL	0.5 mM final concentration
FAD 100μM	1 mL	10 μM final concentration
DTT 200mM	2 100 μL	2 mM
Glycerol	1 mL	

10. Extraction buffer was mixed, with a final volume of 100 mL

1M Phosphate buffer pH 7.5	10 mL	100 mM	final concentration
$MgCl_2$ 6 H_2O 5mM	0.1 g	0.5 mM	final concentration
Sodium pyruvate (200mM)	5 mL	10 mM	final concentration
EDTA Na_4 (0.5M stock)	1 mL	5 mM	final concentration
Valine (fwt. 117.1)	0.012 g	1 mM	final concentration
Luecine (fwt. 131.2)	0.013 g	1 mM	final concentration
DTT (200mM)	1 mL	2 mM	final concentration
FAD (100μM)	10 mL	10 μM	final concentration
TPP	10 mL	0.5 mM	final concentration
Glycerol	10 mL	10 %	final concentration
PVPP (wet wt.)	5 g	5 %	final concentration
Protease inhibitors	6 mL		
XAD-4	4 g		

11. The most recently matured leaves weighing approximately 4.5 g were cut from plants.

12. Mortars were removed from the freezer and sand was placed in bottom of the mortar, followed by the leaves, and then 20 mL of extraction buffer/class of herbicide.
13. The extract was then passed through tissue into Beckman® tubes (kept on ice) through 2x cheese cloth using a glass funnel.
14. Tubes were weighed to ensure they were balanced, then centrifuge at 20,000 g and 4^0 C for 20 min. Beakers were pre-chilled with ice on a stir plate.
15. The supernatant was collected and the volume was measured using a graduated cylinder.
16. PEG 4000 was then slowly poured at 5 % wt/vol., while the extract was stirred on ice for fifteen minutes. After this time the extract was poured into clean tubes.
17. Tubes were weighed again to ensure they were balanced, then centrifuged at 20,000 g and 4^0 C for 20 min.
18. The supernatant was collected and the volume measured, using a clean graduated cylinder.
19. PEG 4000 was added all at once at 20 % wt./vol., while the extract was stirred on ice for fifteen minutes. Then the extract was poured into clean tubes.
20. The tubes were weighed again to ensure they were balanced, then centrifuge at 20,000 g and 4^0 C for 20 min.
21. The supernatant was decanted and the pellet was re-dissolved in re-dissolve buffer, which can be altered to increase or decrease the enzyme content.
22. After the pellet was re-dissolved, the column was pre-equilibrated with 25 mL of buffer containing 10 μM of FAD and 5 mM potassium-phosphate buffer pH 7.5.

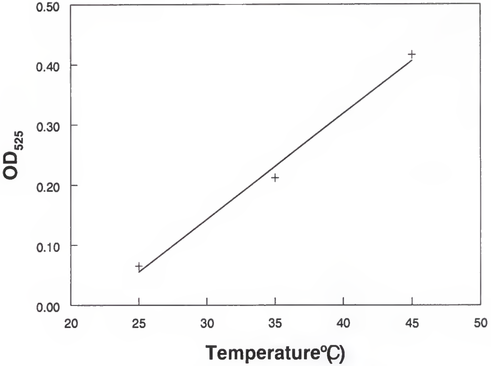
Then 5 mL of re-dissolved pellet was loaded onto Sephadex G-25 columns. All extract was run through columns at 4^0 C.

23. The pump that ran the extract through the column was driven by a Bio-Rad ECONON pump calibrated, using BSA, to pump 1 mL/minute of extract. The elution was collected in 8 mL fractions. Each extract (5 mL) would yield approximately 20 mL of protein extract.

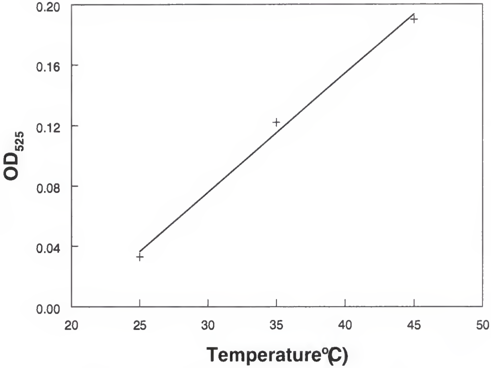
TPP, pyruvate, valine, leucine, and FAD were purchased from Sigma chemical company P.O. Box 1450 St. Louis, MO 63178. PVPP, PEG, and $MgCl_2$ was purchased from Fisher Company Fair Lawn, New Jersey 07410.

APPENDIX C
ALS ASSAY TEMPERATURE

Enzyme used in the temperature assays was extracted as described in APPENDIX B. Once the enzyme content was quantified, 500µg of enzyme was added to each assay mixture and activity was assayed at different temperatures. All assays were conducted simultaneously at 25, 35 and 60°C, by preparing three different water-baths The temperatures were checked before assays were started to ensure proper temperature. All assays were ran for 60 minutes.



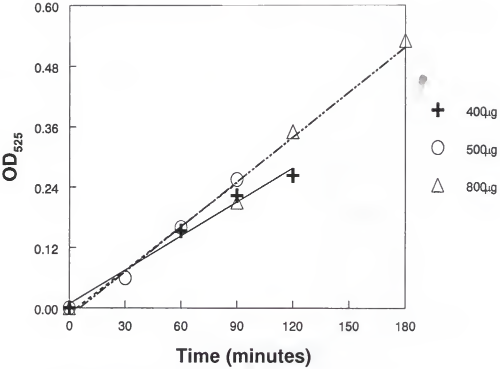
Activity of pepper ALS at varying temperatures.



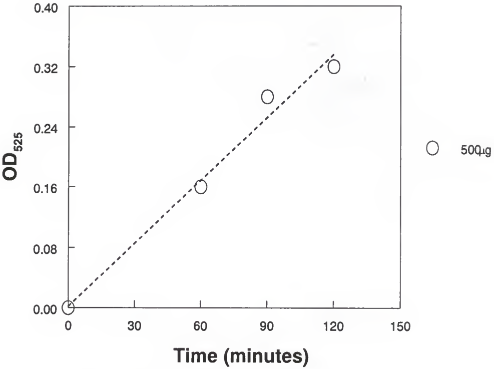
Activity of tomato ALS at varying temperatures.

APPENDIX D
ALS ASSAY TIME

Enzyme used in the experiments investigating optimum time for assaying ALS activity was extracted as described in APPENDIX B. Once the enzyme content was quantified, 400, 500, and 800μg of protein was added to pepper, while 500μg was added to tomato assay mixtures. The objectives were to quantify activity over different time periods. This was accomplished by starting all of the assays at the same time in one water bath and removing them at different time points. The pepper and tomato experiments were conducted over several months, and pepper was assayed with different protein concentrations. The results from all the assays indicated that a 90 minute assay will provide abundant reaction product to detect minor inhibitions from inhibitors. Furthermore, the assay were still in the linear portion of the curve, indicating there was adequate substrates for the reactions to proceed at maximal velocity with no feed back inhibition from reaction products.



Activity of pepper ALS at varying time and ALS content.



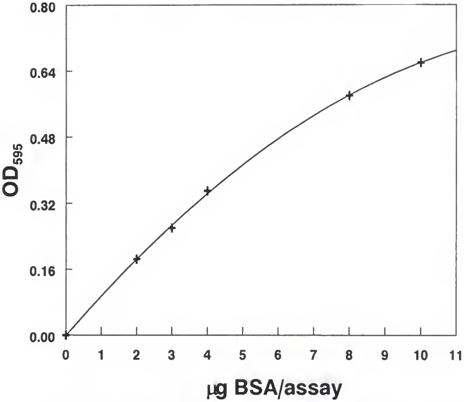
Activity of tomato ALS at varying time and ALS content.

APPENDIX E
ALS ASSAY CONTENT

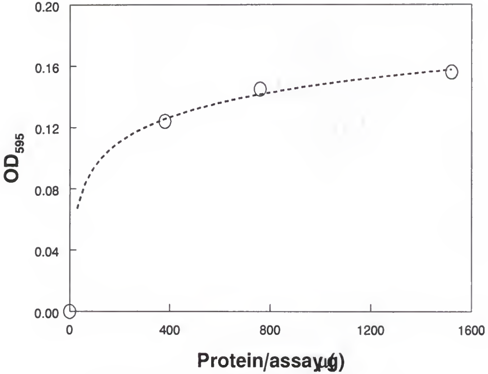
Enzyme used in assays investigating optimum protein concentration for an ALS assay, was extracted as described in APPENDIX B. Once the enzyme content was quantified, 380, 760, and 1520μg of protein was added to tomato, while 200, 400, and 800μg were added to pepper assay mixtures. The objectives were to determine the optimum level of extract to provide the most efficient level of activity, by quantify activity over a range of protein concentrations. This was accomplished by quantifying the protein content in each extraction using the Bradford and Lowry methods, to ensure the level of protein being harvested and added to each assay was accurate. The results from the two assays indicated the Lowry method estimates half the protein estimated by the Bradford method. The differences were consistent over time between the species. All the assays indicated that a 500μg assay will provide optimum activity while conserving the enzyme extract. The Lowry method takes one hour to determine the protein content in the extract, while the Bradford method takes minutes. Therefore, the future assays used Bradford method because of ease. All assays were conducted with 500μg/assay based on the chart below.

Bradford reagent was purchased from Sigma Chemical Company P.O. Box 1450 St. Louis, MO 63178.

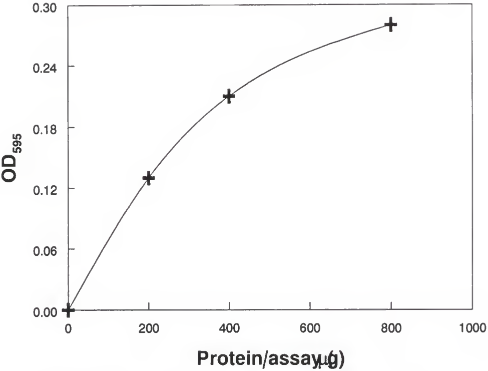
	Protein content in µg/µl	
	Tomato	Pepper
Bradford	11.5	16.5
Lowry	6.25	8.75



Standard curve composed using BSA to estimate protein content.



Activity of tomato ALS at varying protein levels.



Activity of pepper ALS at varying protein levels. Activity was quantified at OD_{525}.

APPENDIX F
ALS ASSAY CONDITIONS

Assay Preparation:

1. All tubes were washed and triple rinsed the day prior to when the assay was conducted.
2. Assay mixture and tubes used in the assay were kept on ice during preparation.
3. Preparing Assay Mixture

Phosphate buffer pH 7.5	200mM final concentration
TPP	5mM final concentration
$MgCl_2$ 6 H_2O	5mM final concentration
FAD	100μM final concentration

Na Pyruvate was added to each assay, however the concentration varied depending on the treatment. This substrate was mixed the day of the assay when kinetic studies were being conducted.

4. Herbicides were mixed the day of the assays. Technical grade rimsulfuron (95% pure) and halosulfuron (98% pure) provided by DuPont and Monsanto, respectively, was used for all assays. One lot of rimsulfuron and halosulfuron were used for all assays conducted. Herbicides used as inhibitors in the kinetic studies were mixed the day of the assay. To achieve accurate concentrations of halosulfuron mixtures, the herbicide was mixed for a concentration of $1x10^{-4}$M.

The total volume was 1.5L Assay mixture was added to the test tubes first, since this contained the buffer. Then the herbicide and substrate (pyruvate) was added, and finally the enzyme extract was added. The final volume of each assay was 400 µL.

5. The reactions in each assay were stopped by adding 50 µL6N H_2SO_4 and heating for 15 minutes at 60° C. Creatine 0.5% was made by mixing 0.125g/25 mL of dd H_2O. α Napthol 5% was made by mixing 1.25g/25 mL of 2.5N NaOH. 500 µL of creatine and α napthol was added to the assay tubes and heated for 15 minutes at 60° C to detect activity. The sensitivity of each assay was increased by allowing each treatment replication to cool at room temperature for 20minutes. Assay mixes were then centrifuged for 1 minute at 14,000 rpm, to removed particles that caused variation in readings.

TPP, creatine, pyruvate, and FAD were purchased from Sigma chemical company P.O. Box 1450 St. Louis, MO 63178.

NaOH, α-Napthol, and $MgCl_2$ was purchased from Fisher Company Fair Lawn, New Jersey 07410.

APPENDIX G
UPTAKE AND TRANSLOCATION

Daily air temperature and average light intensity of the halosulfuron foliar uptake and translocation study.

Date	Maximum (°C)	Minimum (°C)	Light Intensity (µmoles s^{-1})
8/8/2001	45	21	
8/9/2001	44	23	
8/10/2001	41	24	
12/19/2001	33	14	295
12/20/2001	28	20	400
12/21/2001	30	9	290

Average daily air temperature and light intensity of the rimsulfuron root uptake and translocation study.

Date	Maximum (°C)	Minimum (°C)	Light Intensity (μmoles s^{-1})
6/27/2000	37	33	280
6/28/2000	35	35	285
6/29/2000	36	34	270
6/30/2000	37	33	280
7/7/2000	29	27	160
7/8/2000	30	26	169
7/9/2000	28	26	155
7/10/2000	28	27	165

Daily air temperature and light intensity of the halosulfuron root uptake and translocation study.

Date	Maximum (°C)	Minimum (°C)	Light Intensity (μmoles s^{-1})
11/14/2001	44	23	32
11/15/2001	42	16	32
11/16/2001	40	20	32
11/17/2001	39	20	32
12/3/2001	45	11	
12/4/2001	42	15	
12/5/2001	45	18	
12/6/2001	44	16	

Translocation samples were quantified by oxidizing in a R.J. Harvey OX-500. Methyl acrylate standards were run before and after samples. Standards and samples were run for two minutes. Manufacturer recommended settings were used, including maximum sample weight of plants (samples were not more than one gram). The quench curve listed below, was created by burning 15 ^{14}C methyl acrylate standards in the OX-500 biological oxidizer. The 15 standards were counted in the Beckman 6000 LSC to select standards close the theoretical quantity added. Seven were selected, and yellow food coloring was added starting with one drop and increasing one drop to each vial. Dyed vials were then used to create quench curve for the OX-500 oxidizer.

Values of the quench curve used to quantify radioactivity in samples from OX-500.

Standard number	H#	Measured efficiency	Calculated efficiency	Percent difference
1	193	85.07	84.47	-0.7
2	242	76.56	78.28	2.2
3	263	74.05	74.27	0.3
4	285	70.61	69.39	-1.8
5	321	60.98	60.22	-1.3
6	368	46.43	47.06	1.3
7	475	20.4	20.37	-0.2

Quench curve coefficients

A=4.1669774

B=0.0034187

C=--0.000009

D=-0.0000000065

APPENDIX H
RIMSULFURON EXTRACTION AND IDENTIFICATION

1. Enough acetonitrile:water [80:20 buffered with 5mM of potassium-phosphate (pH 7.0)] was mixed for 8 mL/treatment with 5% error. 3.5 g of acid washed sand was then added to each test tube used to grind leaves in.
2. At the end of the incubation period, leaves were cut with a new scalpel one for each species, and placed into screwcap test tubes. Test tubes were placed in G-7 boxes with ice and extracted with 4 mL twice. The blank was extracted first followed by the spike treatment, and then the non-labeled rimsulfuron followed by ^{14}C labeled rimsulfuron treatments. Separate grinding rods were used for the first two extractions (blank and spike) and the last three extractions (non-labeled and ^{14}C labeled rimsulfuron) and for each species.
3. After extraction, using a glass pipette, the solvent was removed from test tubes and centrifuged for 7min at 1,470g. Aqueous fractions were moved into screwcap test tubes with volume marks on side.
4. 100 μL was removed from ^{14}C labeled rimsulfuron treatments to recovery, using LSC.
5. Metabolite extracts were evaporated under N_2 to a final volume of 0.2 mL, while on ice.

Plant samples analyzed directly after extraction, using high performance liquid chromatography, gave many ultraviolet (UV) light peaks from endogenous compounds and was difficult to identify rimsulfuron and rimsulfuron metabolites. Rimsulfuron and its metabolites were further separated from UV absorbing plant residue, using thin layer chromatography (TLC).

1. Silica gel TLC plates (Si250F J.T. Baker chemical company) were prepared for separating samples by running solvent to remove impurities. The solvent was 190:10:2v/v of chloroform:methanol:water. After this was completed plates were air dried.
2. Wells were loaded with 10 µL/cm of tomato and pepper extract using template (wrapped in clean saran). The sides of TLC plate were marked where the well was started. Wells were loaded using a 10 µL pipette man and micro-pipette tips.
3. Below is an example of TLC plate. Ran for ~40 minutes.

4. Movement of rimsulfuron and its metabolites on TLC was tracked by developing audio radiograph. Zones parallel to the radioactive sample were scraped into glass

vials. Rimsulfuron and its metabolites were removed from silica gel, by dissolving in 4 mL of acetonitrile:water and vortexing twice, for 4minutes each time. Efficiency of rimsulfuron extraction from silica gel was monitored using the ^{14}C rimsulfuron lanes.

5. After each vortexing the silica/solvent solution was centrifuged for 7minutes at 1,470 g and moved into vials to evaporate, using N_2 to a final volume of 0.2 mL. Samples were then placed into -80° C until analyzed by HPLC and mas spectrometry.
6. The HPLC used to separate rimsulfuron and its metabolites was a Beckman System Gold with a model 126 pump (Beckman Instruments Fullerton, CA). It was equipped with Phenomenex LUNA C-18 guard column (Torrace, CA) connected to a Waters Symmetry shield RP18, 2.1x150 mm column (Milford, MA). The UV was an Applied Biosystems model 785A programmable absorbance detector, with a wavelength set at 254 nm. The injector was a LCQ's Rheodyne, 25 μL injection loop.
7. The mobile phases of the HPLC were A=10 mM NH_4OAc in H_2O and B=MeOH. Ratio of A:B was initially 95:5 and moved to 5:95, with a flow rate of 0.2 mL/minute.
8. The Mass spectrometry was preformed using a ThermoFinnigan LCQ in electro spray ionization (ESI) and atmospheric pressure chemical ionizations (APCI) modes.

9. The sheath and auxiliary N_2 gas settings for ESI were 60 and 5, respectively. Spray voltage was set at 3.5 kV, while the cap temperature was 250^0 C and voltage was 15. The tube lens offset was 0V.

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BIOGRAPHICAL SKETCH

Richard Buker was born on July 9, 1974, to Robert and Barbara Buker in Ft. Lewis, WA. He first became interested in agriculture and specifically the problems of weeds while growing up in Clewiston, FL. After graduating from Clewiston High School in 1992 he pursued a bachelor's degree at Palm Beach Community College. After attending one year, he transferred to Florida Southern College, where he earned a bachelor's degree in citrus in the spring of 1996. In the fall of 1996, he enrolled at the University of Florida to pursue a Master of Science degree. He received a Master of Science degree from the University of Florida on May 1, 1999, and started to pursue a Doctor of Philosophy degree at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

William M Stall

William M. Stall, Chair
Professor of Horticultural Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

B. Rathinasabapathi

Bala Rathinasabapathi, Cochair
Assistant Professor of Horticultural Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Greg E. MacDonald
Assistant Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Stephen M. Olson

Stephen M. Olson
Professor of Horticultural Sciences

This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 2002

Jimmy G. Cheek

Dean, College of Agricultural and Life Sciences

Dean, Graduate School